SYNTHESIS AND BIOLOGICAL EVALUATION OF OXYGENATED FTORAFUR (FT-207) DERIVATIVES

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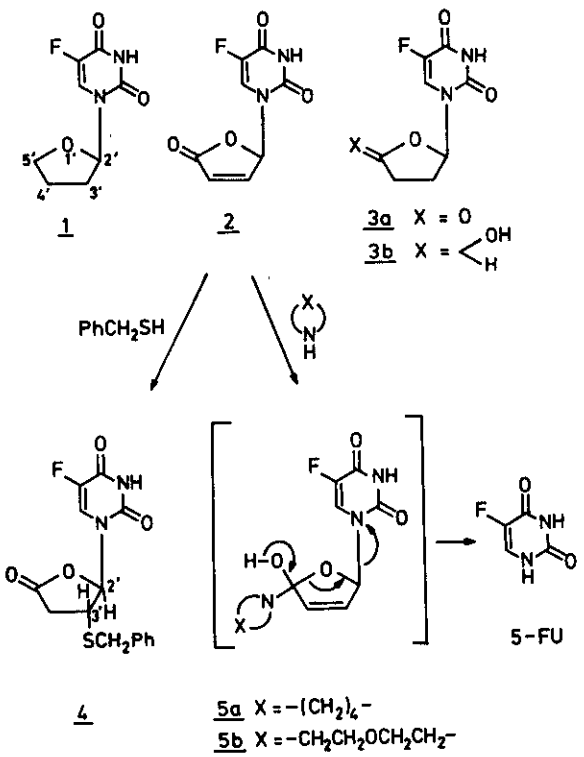
Abstract — The synthesis and the chemical and biological properties of 5'-hydroxy, 5'-oxo and 3'-hydroxy-5'-oxoftorafur are reported.

The effectiveness of Ftorafur 1 (FT-207, 1-(tetrahydrofuran-2-yl)-5-fluorouracil) as an anticancer drug has stimulated new interest in the biological activity of 5-fluorouracil (5-FU) derivatives. As a part of a programme directed to the development of new 5-fluorouridine analogues which could serve as clinically valuable pro-drugs of 5-FU, we have recently reported the synthesis of several non-glycosidic analogues of the nucleoside[1,2]. The observed antileukemic properties (P 388: T/C = 196% at 60 mg/kg) of 5'-oxoftorafur derivative 2, (NSC nr 315845), in particular, have prompted us to undertake the synthesis and biological evaluation of a number of rationally designed derivatives of 2. The results of this study are presented in this communication.

Since it is well established that cytotoxic activity in a molecule can be correlated with the presence of a reactive electrophilic double bond[3,4], great interest was attached to the biological evaluation of the saturated lactone corresponding to 2, namely, compound 3a. In the same context, reactions of 2 with nucleophiles bearing functional groups present in proteins, were recognized as a pertinent area of investigation. Furthermore, it was felt that derivatives of 2 which were related to active metabolites of ftorafur formed valuable target compounds.

In order to investigate whether the unsaturated lactone moiety was essential to the activity of 2, the compound was catalytically reduced (H_2/Pd-C) to the dihydroproduct 3a, mp 204°-207° C[5], (76%). This saturated lactone was, however, inactive in the P-388 test system. This result suggests that the unsaturated lactone functionality is indeed necessary for the cytostatic activity of 2.

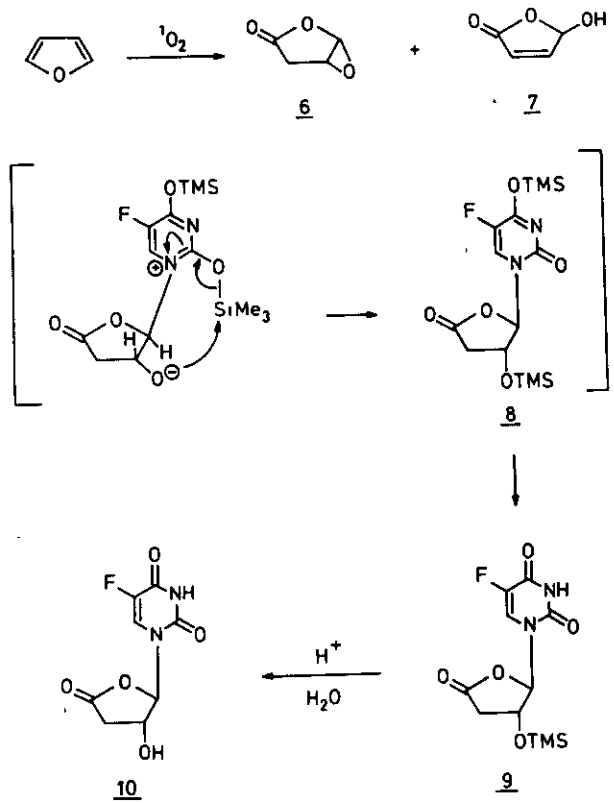
For the study of the reactions of 2 with nucleophiles, suitable amines and thiols were chosen.



1 2 3a X = O 3b X = OH H

4 5a X = −(CH2)4− 5b X = −CH2CH2OCH2CH2−

Scheme I



6 7 8 9 10

Scheme II

With secondary amines such as pyrrolidine and morpholine, the reaction led to a rapid formation of 5-FU. With benzyl-thiol on the other hand, the Michael adduct 4 was isolated as a crystalline product. The structure of 4 followed from its ^{1}H NMR spectrum[6]. The trans relationship between the nucleobase and the thio ether group in 4 was attested by the coupling constant between the H-2' and H-3' protons[7] ($J \geq 6$). This stereochemistry is the result of the expected course of attack of the thiol group on the lactone ring from the side opposite to the 5-FU moiety.

The difference in the patterns of the nucleophilic reactions of amines and benzyl thiol with 2, can be rationalized as follows. The unsaturated lactone in 2 possesses two electrophilic centres, namely the C-5' carbonyl carbon and the C-3' end of the olefinic linkage. The relatively harder nucleophilic amines attack as expected the (hard) carbonyl group, to initially give intermediates 5a,b. These shall, however, fragment to release 5-FU in a facile process. In contrast, the softer thiol nucleophile attacks the soft olefinic centre[8,9] to give the isolable adduct 4.

In view of the known addition of a cysteinyl mercapto group to the 5,6-double bond of 5-fluorodeoxyuridine monophosphate[10,11], during the inhibition of thymidylate synthetase by this fluoro nucleotide, the reaction of 2 and 4 with excess of benzyl thiol was investigated under different reaction conditions. However, in none of the reactions an addition of the thiol to the double bond was observed.

Investigation of the metabolism of ftorafur (1) has revealed that while 3'- and 4'-hydroxy derivatives of 1 can be isolated, these metabolites are biologically incompetent[12-16]. The 2'- and 5'-hydroxylated derivatives of 1, on the other hand, if formed, would be capable of releasing 5-FU. The finding by Sadeé and coworkers that 1 is hydroxylated at C-5' by microsomal cytochrome P-450[17-19] is consequently, highly significant. Despite the anticipated instability of 5'-hydroxyftorafur (3b, Scheme I), attempts were made to prepare this metabolite by low temperature reductions of 3a with the DIBAL reagent[20]. The only isolable product of the reduction reactions was 5-FU. We suggest that these results are consistent with the formation of 3b, and that subsequent decomposition in a manner analogous to the fragmentation of 5a,b occurs in accordance with the proposal put forward by Sadeé[17,19].

The hydroxylation of the lactone ring of 3a at the 3'-position has been successfully achieved by the sequence of reactions described in Scheme II. The starting material for the synthesis was epoxylactone 6, which was obtained as a minor product (5%), along with 7 (80%), by the oxidation of furan with singlet oxygen[21]. When 6 was coupled to a 5-FU moiety by reaction with 2,4-bistrimethylsilyloxy-5-fluoropyrimidine ($SnCl_4$, -40°); hydrolysis of the reaction mixture led to the formation of 9[22] (40%) as a crystalline product, mp 194-196°C. Presumably, during the Hilbert-Johnson type reaction of 6 leading to 8, the attack of pyrimidine on the epoxy carbon is followed by an intramolecular transfer of the trimethylsilyl group to the alkoxide ion generated in the

first step. Removal of the silyl ether function of 9, under mild conditions (acetic acid/water/acetone, RT) gave the 3-hydroxy-5-oxoftorafur 10[23], in 60% yield.

BIOLOGICAL RESULTS

On the basis of the biological activity found in the P-388 leukemia test-system, 2 was selected by NCI for a wide-panel tumour screening and prepared by us on a 50 g scale. Antileukemic activity was confirmed in the L-1210 system (T/C = 140-160% at 50 mg/kg). Results on solid tumour test-systems however were disappointing. On CX-1 human colon xenograft in nude mice, reduction to 47% of tumour weight was found at 150 mg/kg. No significant activity was observed on MX-1 mammary xenograft, CD mammary tumour, Colon-38, Lewis-lungcarcinoma and B-16 melanoma. In P-388 test system, no activity was found for compounds 3a and 9.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. G. Atassi, Institut Jules Bordet, University of Brussels for cytostatic screening and to the Koningin Wilhelmina Fonds (The Netherlands Foundation for Cancer Research) for financial support.

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5. 3a: Mp: 204-207° C (76%). IR (KBr): 3170, 1800, 1710/1695, 1650 cm^{-1}. ^1H NMR (d_6-DMSO): δ 2.2 - 2.8 (m, CH_2-CH_2); 6.41(dxd, O-C-N); 8.25(d, J=7, =C-H); 11.9 (NH).

6. 4: Mp: 100°C (dec.); IR(KBr): 1780, 1730, 1700, 1670 cm^{-1}; ^1H NMR (d_6-DMSO): δ 2.75, 3.12(AB x d, J=18, J=6, J=6, CH_2): 3.6-3.8("q", J=6, HCS); 3.82(s, CH_2,Ø); 6.31 (d, J=6, OCHN); 7.13 (s, Ø); 8.15 (d, J=7, =C-H).

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22. 9: Mp: 194-196°C (40%), IR(KBr): 3470, 1788, 1705, 1261 cm^{-1}; ^1H NMR (d_6-DMSO): δ 0.1(s, $SiMe_3$); 2.45, 3.25(AB x d, J=18, J=8, J=4.5, CH_2); 4.8 (m, H-C-O); 5.95(d, J=3, O-CH-N); 8.04(d, J=7, =C-H).

23. 10: Mp: 183-185° C (60%); IR(KBr): 3470, 1788, 1705. ^1H NMR (d_6-DMSO): δ 2.55, 3.20 (AB x d, J=18, J=8, J=5.5, CH_2); 4.7(m → "quintet" with D_2O, H-C-O); 6.03(d, J=4, O-CH-N); 8.31(d, J=7, =CH); 12.1 (broad, NH).

Received, 23rd July, 1984