# POTENTIAL CARCINOSTATIC AGENTS.[1] PSEUDO-GLYCOSIDIC[+] DERIVATIVES OF 5-FLUOROURACIL.

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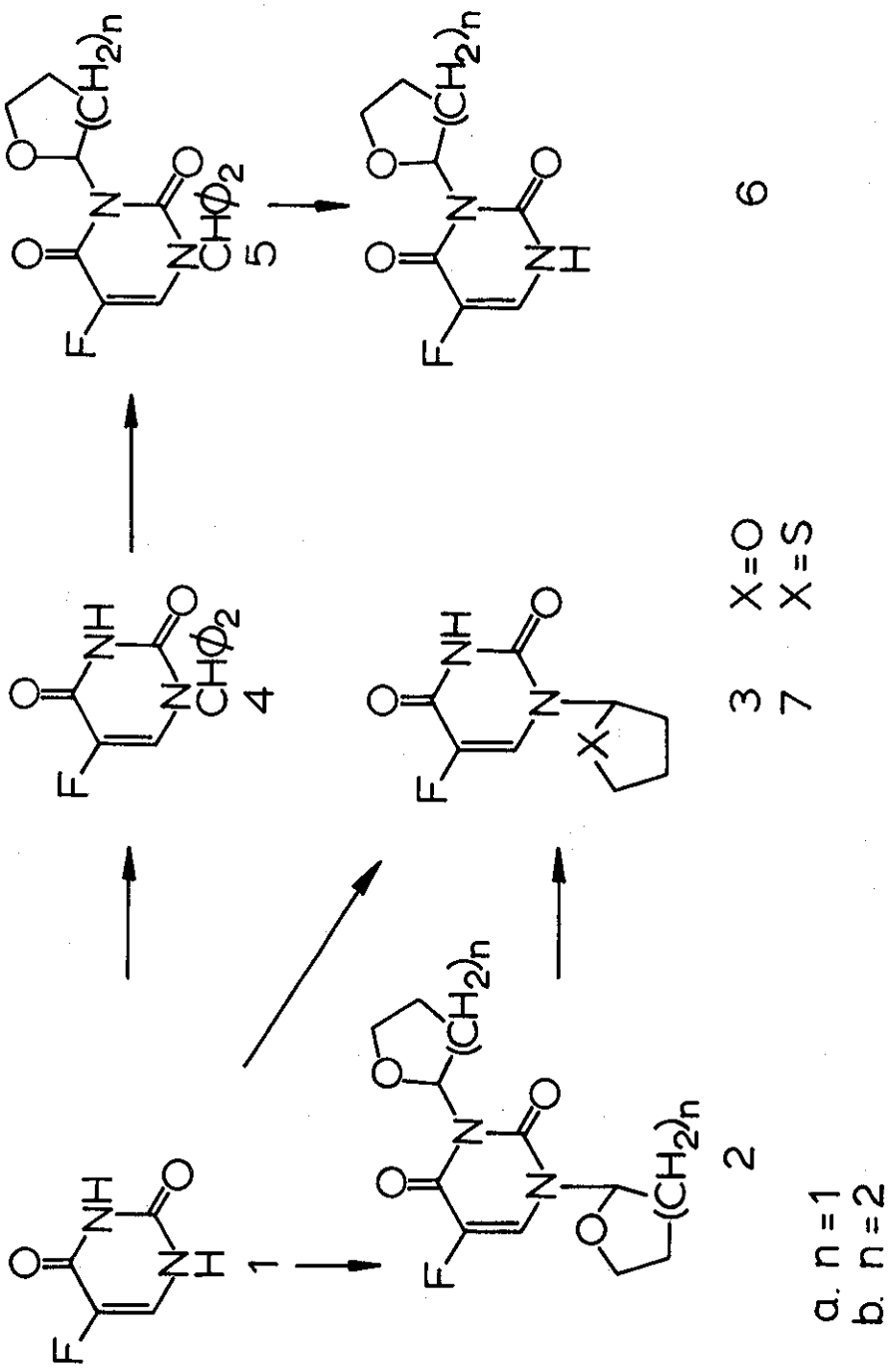
The synthesis and carcinostatic properties of pyranyl-, tetrahydrofuryl- and tetrahydrothienyl-derivatives of 5-fluorouracil are described.

Since the first report on the synthesis and chemotherapeutic properties of 1-(tetrahydrofuran-2-yl)-5-fluorouracil (Ftorafur[2], 3a), the compound has received a great deal of attention as a carcinostatic agent in the treatment of malignant tumors of the breast and the gastro-intestinal tract[3,4]. The reported low toxicity and long-lasting action of Ftorafur raises several questions with regard to the mode of action of the drug. Thus far it has been generally assumed that the properties of Ftorafur are not due to the intact nucleoside analogue but that the latter serves as a repository form of 5-fluorouracil (1) which is released prior to or after the passage of the drug (3a) across the cell-membrane[2,5]. Meiren and Belousova[6] report that Ftorafur (3a) does not split off 5-fluorouracil (1) under influence of nucleoside phosphorylase, as suggested by Hiller et al.[2]

[+] A pseudo-glycoside is defined as a system in which the nucleobase is attached, via one of its nitrogens, to a -C-OR-moiety.

Recently, Wu et al.[7] observed the formation of hydroxylated metabolites of Ftorafur in rats and rabbits. In order to derive information on the mechanism of action of 3a, we have undertaken a study[8] of the synthesis and biological evaluation of suitable derivatives of 1, which can release the fluoropyrimidine (5-FU, 1) under physiological conditions at different rates. The recent report on the reaction of 1 with 2-alkoxytetrahydrofurans, by Kametani et al.[9], prompts us to communicate our preliminary results on the preparation and properties of the pseudo-glycosides of general structures 2 and 6.

When 1 was allowed to react with an excess of 2-chlorotetrahydrofuran[10] ($CH_2Cl_2$, $Et_3N$), 1,3-di-(tetrahydrofuran-2-yl)-5-fluorouracil (2a) was obtained as a crystalline product (m.p. 103-106°[9], $\delta$ $H_1$',=6.58 and 5.95, m) in practical yields. Since there is a considerable difference in the stabilities towards hydrolytic conditions, between the $N_1$- and $N_3$-substituted 5-fluorouracil derivatives[11], compound 2a serves as a valuable precursor of Ftorafur. Thus, the conversion of 2a to 3a can be readily achieved by treatment of the former with aqueous HCl-ethanol (pH 5). Reaction of 1 with one equivalent of 2-chlorotetrahydrofuran leads to 3a; however, the product is always contaminated with small amounts of 2a. These results are in agreement with the observations of Kametani et al.[9]. With 2-chlorotetrahydropyran (prepared via addition of gaseous hydrogen chloride to 2,3-dihydropyran), similar results were obtained. For the preparation of the tetrahydrothienyl derivative 7a, a solution of 5-fluorouracil in DMF was treated with 2-acetoxytetrahydrothiophene[12] at 150°. The product was obtained in pure crystalline


1
2
a. n=1
b. n=2
3 X=O
7 X=S
4
5
6

form from methanol (m.p. 193-195°, $\delta_{H_1}$,= 6.7 m.)

Since 1 cannot be substituted at the $N_3$-position directly, for the synthesis of 6a ("iso-Ftorafur") and 6b, the following approach was employed. 5-Fluorouracil (1) was converted into its anion (NaH/HMPT) and the latter allowed to react (HMPT, 100°) with benzhydryl chloride, whereupon 1-benzhydryl-5-fluorouracil (4), m.p. 169-170°, was obtained (40%). The protected uracil derivative could be conveniently alkylated with the chloro-ethers according to the conditions described above. Removal of the benzhydryl group proved to be highly sensitive to the hydrogenation procedure. Best results were obtained when a dioxane solution of 5a was reduced over palladium catalyst (3-4 atm.) for 24 h. (6a: m.p. 133-134° C, $\delta_{H_1}$,=6.55, m). Longer reaction times caused a cleavage of the furanyl group. The more stable pyranyl derivative 5b was reduced in ethanol/ethylacetate using the same catalyst and hydrogen pressure. (6b: m.p. 151-153°, $\delta_{H_1}$,=5.85 d × d).

Biological results.

The uptake of $^3$H-thymidine by 3T3-F cells[13] in in vitro culture was stimulated by both 6a and 6b. This result indicates inhibition of thymidylate synthetase by these derivatives. Whether this activity is due to interaction of the enzyme with 6a and 6b, as intact molecules or to 5-fluorouracil after hydrolysis could not be established unequivocally.

In vivo screenings were carried out both on leukemia P-388 and sarcoma S-180 test systems. The available results are summarized in the Table.

TABLE

| Compound | NSC no | dose ($^{mg}/kg$) | P-388 $^{T}/C^{a}$ | S-180 $^{C}/T^{b}$ |
|---|---|---|---|---|
| 2a | 288544 | 150 | 96 | |
| | | 75 | 100 | |
| | | 37.5 | 103 | |
| 6a | 288543 | 100 | 156 | |
| | | 50 | 147 | |
| | | 25 | 123 | |
| 6b | 297390 | 100 | 218 | $1.5^{c}$ |
| | | 50 | 176 | $1.7^{c}$ |
| | | 25 | 159 | - |
| 7 | | 200 | | $2.0^{c}$ |
| | | 100 | | $1.2^{c}$ |

[a] Carcinostatic activity expressed as % survival time relative to untreated control animals. T/C > 125% is a criterium for activity.

[b] $C/T = \frac{\text{tumor weight of controls}}{\text{tumor weight of treated animals.}}$

[c] At the concentrations used, no significant leucopenia was observed.

Further examination of the abovementioned compounds and the synthesis and biological evaluation of a variety of pseudo-glycosides of 1 is in progress.

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