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# Intraocular drug delivery. In vitro release studies of 5-fluorouracil from $N_1$-alkoxycarbonyl prodrugs in silicone oil

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**Abstract**

Various $N_1$-alkoxycarbonyl prodrugs of 5-fluorouracil (5-FU) ranging from 5 to 18 carbon units in the pro-moiety were synthesized. The compounds were physico-chemically characterized by determining the ionization constant ($K_a$), aqueous solubility ($S$), octanol/water partition coefficient ($P$), and the degradation rate in aqueous solution at different pH values. The in vitro degradation rate of the prodrugs in calf serum, human plasma, and rabbit vitreous humor was also investigated. Drug delivery systems were prepared by dissolving or dispersing 5-FU prodrugs in silicone oil 1000 (SO-1000). The release of 5-FU seemed to follow the square root of time kinetics until more than 60% of the drug had been released. The release rate of 5-FU was found to decrease with increased lipophilicity of the prodrug.

*Keywords:* 5-Fluorouracil; Prodrugs; Silicone oil 1000; Controlled release; Ocular drug delivery

## 1. Introduction

Proliferative vitreoretinopathy (PVR) is characterized by a rapid and uncontrolled proliferation of ocular cells within the vitreous body of the eye frequently resulting in retinal detachment (Glaser et al., 1987; Machmer, 1988). Intravitreal injection of silicone oil may successfully reattach the retina, although failures due to late reproliferation are common (Lucke and Laqua, 1990). Pharmacological studies have shown that the antimetabolite 5-FU inhibits growth of rabbit fibroblasts both in vitro and in vivo as well as being capable of reducing experimental intraocular proliferation in rabbits (Stern et al., 1983; Blumenkranz et al., 1984a). Toxicity studies demonstrate that 1 mg 5-FU can be safely administered to humans by intravitreal injection (Blumenkranz et al., 1984b); however, it is rapidly eliminated ($t_{1/2} < 3.5$ h) following intravitreal injection. This phenomenon is particularly notable in cases where the eye has been vitrectomized (the vitreous body is removed) (Rootman et al., 1979; Jarus et al., 1985). Intraoc-

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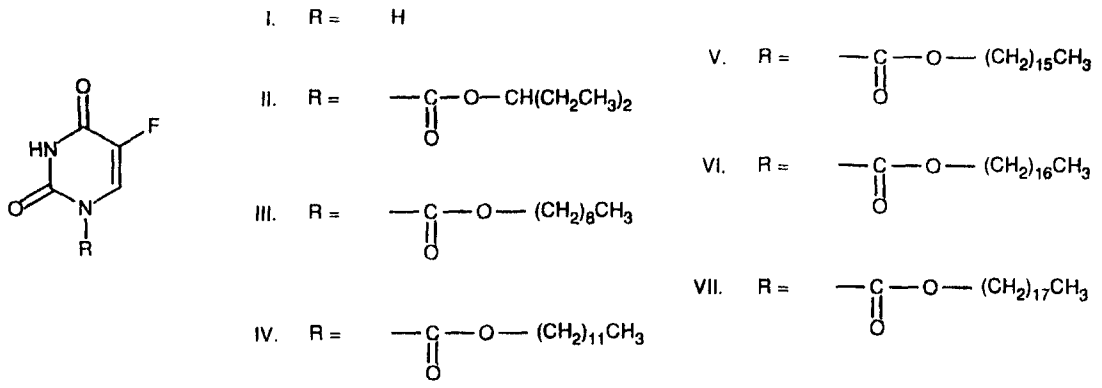


Fig. 1. Long chain $N_1$-alkoxycarbonyl prodrugs of 5-fluorouracil.

ular therapeutic levels of 5-FU over several days, which are needed in the treatment of PVR, are restricted due to irreversible retinal and corneal toxicity caused by high peak levels within the vitreous cavity (Capone et al., 1987; Leon et al., 1990). A combination of silicone oil therapy and a slow intraocular release of 5-FU, maintaining therapeutic levels for several days, may be helpful in the treatment of extreme PVR (Hartzer and Blumenkranz, 1988). A promising approach may include development of drug delivery systems of 5-FU prodrugs in silicone oil which can maintain therapeutic levels of 5-FU over several days.

## 2. Materials and methods

### 2.1. Equipment

Elemental analysis was performed at the Microanalytical Laboratory, University of Copenhagen, Denmark, and $^{H}$NMR spectra were performed at the University of Mainz, Germany. Readings of pH were carried out on a Radiometer type PHM 83 Autocal meter. UV spectrophotometry was performed with a Shimadzu UV-190 double beam spectrophotometer equipped with a thermostated cell compartment using 1-cm quartz cuvettes. High performance liquid chromatography (HPLC) was performed with a reversed phase isocratic system consisting of a Hitachi-Merck L6200 Intelligent pump, a Hitachi-Merck L-4000 variable wavelength UV-detector operated at 266 nm, a Hitachi-Merck AS-2000 autosampler injecting 25-$\mu$l samples and a Hitachi-Merck D2520 GPC integrator. A Supelcosil C-8 column (150 × 4.6 mm, 5-$\mu$m particles) was used in all measurements except for the determination of 5-FU when an Axxion C-18 column (250 × 4.6 mm, 5-$\mu$m particles) was used.

### 2.2. Chemical and biological materials

5-FU was purchased from Sigma Chemical Co., St. Louis, MO, and was used as received. Silicone oil 1000 was kindly donated by Richard James Inc., USA. Buffer substances and all other chemicals or solvents used were of either HPLC or reagent grade. Bovine calf serum was purchased from Sigma. Human plasma was kindly donated by the State University Hospital, Copenhagen, Denmark. Rabbit eyes were kindly donated by Novo Nordisk, Bagsvaerd, Denmark.

### 2.3. Synthesis

$N_1$-Alkoxycarbonyl-5-fluorouracils were prepared by reacting 5-FU with the appropriate chloroformate prepared from the corresponding alcohol (British Patent, 1977; Buur and Bundgaard, 1986; Steffansen et al., 1991). The solid obtained was purified by recrystallization from ethylacetate/light petroleum. Elemental-analysis and $^{H}$NMR spectrometry was in agreement with the proposed structures. Melting points

were determined in capillary tubes and are uncorrected (Table 2). The chemical structures of the various 5-FU prodrugs are shown in Fig. 1.

### 2.4. Kinetic measurements

All kinetic studies were carried out in 0.05 M aqueous buffer solution at 37.0 ± 0.2°C. A constant ionic strength ($\mu$) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The buffers were HCl (pH ≤ 2), acetate (pH 4–5), phosphate (pH 2–3 and 6–7.4), borate (pH 8.5–10), carbonate (pH 10.5–11.5), and sodium hydroxide (pH 12). Quantification was done by measuring the peak areas in relation to those of standards analyzed under the same conditions. The reactions were initiated by adding 25–100 $\mu$l of a stock solution of each compound, in acetonitrile, to 10–25 ml of a buffer solution pre-equilibrated at 37°C in screw-capped test tubes, the final concentration being $10^{-6}$–$10^{-4}$ M. The solutions were kept at 37°C in a water bath and at appropriate times, samples were taken and assayed. For the determination of the prodrugs, a mobile phase system consisting of $CH_3CN/CH_3OH$/0.1% phosphoric acid, 10–60:30:10–60 was used, the flow rate being 1.0 ml/min. The column effluent was monitored at 266 nm. The detection limit of each compound was calculated to be approximately 0.3 $\mu$g/ml with a signal-noise ratio of 2. 5-FU was determined by HPLC using a mobile phase of 0.02 M acetate buffer at pH 4.0, a flow rate of 1.0 ml/min and a detection wavelength of 266 nm. The detection limit was calculated to be approximately 20 ng/ml with a signal-noise ratio of 2. Pseudo-first order rate constants for the hydrolysis were determined from slopes of linear plots of the logarithm of residual prodrug versus time.

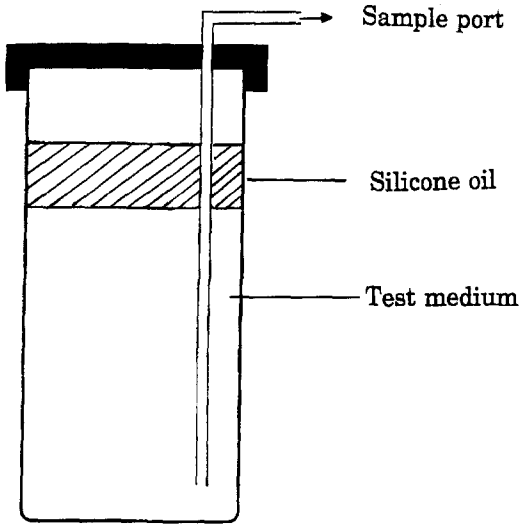


Fig. 2. The in vitro release model. Silicone oil 1000 containing prodrug on top of the test medium.

Biological homogenates were prepared as follows: New Zealand white rabbits were sacrificed by a phenobarbital overdose. Vitreous bodies were isolated and homogenized in 0.02 M phosphate buffer at pH 7.4 to provide a 1:1 (w/v) dilution using a Cowie® glass homogenizer. Human plasma and calf serum solutions of 80% were prepared in 0.02 M phosphate buffer of pH 7.4. The degradation rate of the prodrugs was studied in the various biological homogenates. The initial concentration of the prodrugs was $10^{-6}$–$10^{-4}$ M. At appropriate times, 250 $\mu$l of the biological homogenate was withdrawn and added to 500 $\mu$l of a 0.2 w/v% solution of zinc sulphate in 50% v/v% methanol/water in order to deproteinize the sample. After mixing and centrifuging for 5 min at 13 000 rev./min, 25 $\mu$l of the clear supernatant was analyzed by HPLC as described above. The first-order rate constants ($k_{obs}$) were determined from linear plots of logarithm of residual prodrug versus time.

### 2.5. Aqueous solubilities, partition coefficients, and ionization constants

The intrinsic, aqueous solubilities of compounds II, III and IV were determined at 22°C by adding excess amounts of prodrug to 0.05 M acetate buffer of pH 4.0. The mixture was placed in an ultrasonic bath for 15 min, rotated mechanically for 1 h and subsequently filtered through a 0.45-$\mu$m Millipore® HV filter. The clear filtrate was assayed by HPLC as described above. The solubility of compounds V, VI and VII was not determined as the solubilities were lower than the detection limit of the compound, i.e. < 0.3 $\mu$g/ml.

The intrinsic partition coefficient ($P$) for compounds II, III and IV was determined in octanol/0.02 M acetate buffer (pH 4.0) systems. In order to minimize the degradation, the mixtures were shaken vigorously for only 5 min. Due to the low solubility of the compounds, the partition coefficient of compounds V, VI and VII was not determined but estimated from Eq. (1):

$$\log P_n = a \cdot n \quad (1)$$

where $P_n$ is the estimated partition coefficient for a $N_1$-alkoxycarbonyl prodrug containing $n$ carbon units in the side chain, and $a$ is the slope of linear plots of $n$ versus the logarithm of experimentally determined partition coefficients. Using experimental data from compounds II, III and IV determined above together with experimental data of $N_1$-methoxycarbonyl-, $N_1$-ethoxycarbonyl- and $N_1$-butoxycarbonyl-derivatives of 5-FU reported by Buur and Bundgaard (1986), $a$ was estimated to 0.334 ($r = 0.990$, $n = 6$).

The ionization constants of the $N_1$-alkoxycarbonyl derivatives II, III and IV were determined at 22°C and an ionic strength ($\mu$) = 0.5 by spectrophotometry, using information from the UV-spectrum changes upon dissociation of the 3NH proton (Albert and Serjeant, 1971; Buur and Bundgaard, 1986). The wavelength used for determination of the $pK_a$ values was 235 nm. The solute concentration was $1 \times 10^{-5}$ M and the UV absorbances were measured at pH 2–9.5 and at several different pH values within the range of pH 6–8.

### 2.6. Drug delivery systems for 5-FU prodrugs in silicone oil 1000 (SO-1000)

A stock solution of each prodrug in ethyl acetate was made and 0.5–3.0 ml was added to 50 g SO-1000. The ethyl acetate was removed under reduced pressure, the drug delivery system containing 50–60 $\mu$g equivalent 5-FU per g SO-1000. In these concentrations compound II and III formed clear solutions, whereas IV, V, VI and VII formed turbid suspensions. The particle size in the turbid suspensions was not characterized.

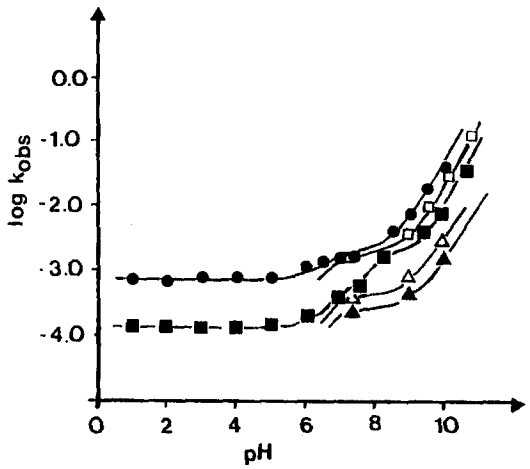


Fig. 3. pH-rate profiles of compounds II (■), III (●), IV (□), V (△), and VI (▲) (37°C and $\mu$ = 0.5).

### 2.7. In vitro release studies

As illustrated in Fig. 2, 1.5 g of the drug delivery system, described above, was positioned on top of 10 ml receptor medium of either phosphate buffer pH 7.40, carbonate buffer pH 10.85, or 80% calf serum. The buffer/SO-1000 interface was 133 mm$^2$. The system was kept at 37°C and 500-$\mu$l samples were periodically taken from the aqueous receptor phase and immediately assayed by HPLC as described above. In order to avoid a concentration gradient in the receptor phase, air was manually pumped through the sampling port by a syringe prior to sampling. Then the sample volume was replaced with the corresponding receptor medium and finally air was pumped through the sampling port. The accumulated released 5-FU was calculated from Eq. (2); where $Q_t$ is the total amount of 5-FU released at time $t$; $V_s$ is the sampling volume; $C_1$, $C_2 \ldots C_n$ is the concentration of sample 1, 2...$n$; and $V_t$ is the volume of the receptor phase. The whole receptor phase was replaced every second day in order to keep it non-infected. The release rate of 5-FU was determined as linear plots of 5-FU released versus the square root of time (Steffansen et al., 1992).

$$Q_t = V_s\left(\sum C_{n-1}\right) + C_n V_t \quad (2)$$

Table 1
Dissociation constants (22°C) and second-order rate constants for hydrolysis of various long chain $N_1$-alkoxycarbonyl derivatives of 5-fluorouracil (37°C)

| Compound | $k_0$ ($min^{-1}$) | $k_{OH}$ ($M^{-1}$ $min^{-1}$) | $k'_{OH}$ ($M^{-1}$ $min^{-1}$) | $pK_a$ |
|---|---|---|---|---|
| II | $1.2 \times 10^{-4}$ | $3.0 \times 10^3$ | 35 | 6.8 (0.01) |
| III | $7.0 \times 10^{-4}$ | $1.7 \times 10^4$ | 230 | 6.8 (0.05) |
| IV | NM | $1.2 \times 10^4$ | 140 | 6.7 (0.1) |
| V | NM | $3.3 \times 10^3$ | 5 | NM |
| VI | NM | $2.5 \times 10^3$ | 1.5 | NM |

NM, not measured.

## 3. Results and discussions

### 3.1. Kinetic measurements

In agreement with previous findings for shorter chain $N_1$-alkoxycarbonyl 5-FU derivatives, the prodrugs were all found to hydrolyze quantitatively to the parent 5-FU in aqueous solution (Buur and Bundgaard, 1986). The kinetics of hydrolysis was studied over a wide range of pH at 37°C. At constant pH, the reactions displayed first-order kinetics for several *half-lives*. The influence of pH on the rate of hydrolysis is shown in Fig. 3, in which the logarithm of the observed pseudo first-order rate constant ($k_{obs}$) is plotted against pH. The derivatives are NH-acidic compounds dissociating at the 3NH proton. Thus, in the pH range investigated, they exist in neutral and anionic forms. The shape of the pH–rate profiles is similar to those for shorter chain $N_1$-alkoxycarbonyl 5-FU prodrugs and can thus be accounted for in terms of specific base-catalyzed reactions of the undissociated ($k_{OH}$) and anionic ($k'_{OH}$) forms together with a spontaneous (pH independent) or water-catalyzed reaction of the undissociated ($k_0$) form (Buur and Bundgaard, 1986). The various rate constants are listed in Table 1 together with their dissociation constants. The branched derivative II is less reactive compared to corresponding $N_1$-alkoxycarbonyl derivatives; yet, the long chain derivatives, as represented by compounds IV and VI, show a decreased reactivity equal to or even less than that of compound II.

$N_1$-Alkoxycarbonyl derivatives undergo enzymatic hydrolysis in 80% calf serum, 80% human plasma and 50% rabbit vitreous at 37°C (Table 2). The hydrolysis of the derivatives follows first-order kinetics and 5-FU is formed quantitatively. It is noteworthy that the formation of parent 5-FU from the derivatives can be explained by a high degree of enzymatic catalysis even for longer chain derivatives. The *half-lives* of these compounds in calf-serum are less than 3 min, which is in sharp contrast to about 30 h in aqueous buffer of pH 7.4. On this basis, it can be assumed that the derivatives act as true prodrugs of 5-FU in vivo.

### 3.2. Aqueous solubilities, partition coefficients, and ionization constants

The intrinsic solubility ($S$) and partition coefficient ($P$) of compounds II, III and IV were determined at pH 4.0 in order to ensure that the compounds were entirely unionized. The data are given in Table 2. Due to the low solubility of the compounds V, VI and VII, their partition coefficients were estimated on the basis of Eq. (1). The calculated values are in reasonably good agreement with values estimated on the basis of the experimental value for compound III and the Hansch substitution parameter ($\pi$), adding 0.5 for each additional methylene group (Hansch and Leo, 1979). As expected, the derivatives are all much more lipophilic compared to parent 5-FU, which has a log $P$ value of $-0.83$ (Buur and Bundgaard, 1986). Furthermore, as the melting points of the derivatives are very much alike, an inverse relationship is found between lipophilicity and aqueous solubility.

Table 2
Half-lives ($t_{1/2}$) for the hydrolysis of $N_1$-alkoxycarbonyl derivatives of 5-fluorouracil in various media, together with partition coefficients ($P$), aqueous solubility ($S$) and melting points

| Compound (molecular weight) | Melting point (°C) | $t_{1/2}$ | | | | Log $P$ octanol/buffer at pH 4.0 | $S$ pH 4.0 ($\mu$g/ml) |
|---|---|---|---|---|---|---|---|
| | | 50% rabbit vitreous body fluid (min) | 80% human plasma (min) | 80% calf serum (min) | Buffer at pH 7.4 (h) | | |
| II (244.22) | 96–98 | NM | 10 | 2.9 | 28.4 | 1.3 | 2900 |
| III (300.33) | 84–85 | <1 | <1 | 2.7 | 5.6 | 2.0 | 17.4 |
| IV (342.41) | 88–89 | <1 | <1 | 2.7 | 7.5 | 3.3 | 9.0 |
| V (398.51) | 98–99 | NM | NM | 2.8 | 27 | 5.4[a] | NM |
| VI (412.54) | 98–99 | 120 | NM | 2.6 | 30 | 5.7[a] | NM |
| VII (426.57) | 94–95 | NM | NM | 2.9 | 31 | 6.1[a] | NM |

NM, not measured.
[a]Estimated values.

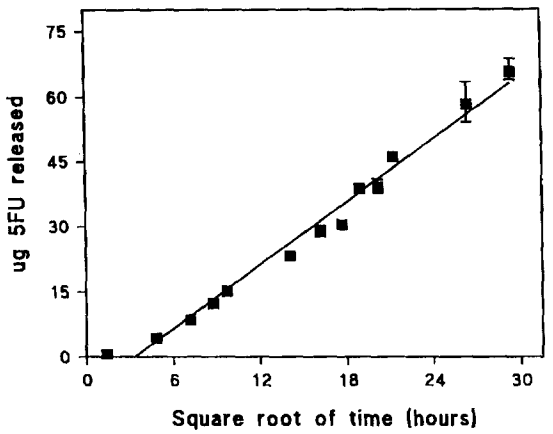


Fig. 4. Release of 5-fluorouracil from compound IV in silicone oil 1000, as a function of the square root of time ($n = 5$, $r = 0.998$).

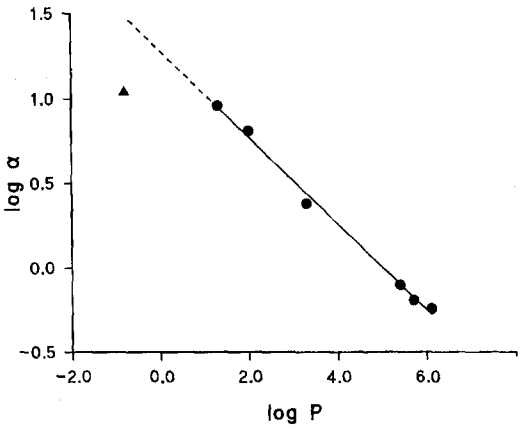


Fig. 5. Log α as a function of log *P*.

The $pK_a$ values of compounds II, III, and IV are listed in Table 1 and agree well with those determined for shorter chain $N_1$-alkoxycarbonyl derivatives of 5-FU (Buur and Bundgaard, 1986).

### 3.3. Release studies

The prodrugs are stable in SO-1000 for the 6 months investigated (Steffansen, unpublished data). The release of 5-FU from the drug delivery system seems to follow square-root-of-time kinetics for the first 60% released, as shown in Fig. 4 for compound IV. For compounds IV, V, VI and VII which formed turbid suspensions in SO-1000 at the concentration investigated a growing depletion zone in the donor phase, starting at the interface could be observed during the experiment. This observation supports the theory that the release rate of 5-FU follows square-root of-time kinetics (Higuchi, 1961). The release rate constant (α), determined as the slope from the linear part of these plots, is listed in Table 3. Negligible amounts of prodrug were found in the receptor phase. For the less lipophilic compounds II and III, it appears that the release rate constant is dependent on the receptor phase. By increasing pH from 7.4 to 10.8 and thereby increasing the degradation rate of the prodrug in the receptor phase, the release rate of compound III increases from 6.5 to 8.5. In contrast, for the more lipophilic prodrugs IV and V, the 5-FU release rate constant is apparently independent of the receptor phase and, thereby, of the hydrolysis rate of the prodrug. Although the release of 5-FU from the drug delivery system is very complex, a key factor for the 5-FU release rate is the lipophilicity of the prodrugs. This is illustrated in Fig. 5, in which the logarithm of the release rate constant is plotted against log *P*. The 5-FU prodrugs all fit a linear correlation, whereas parent 5-FU apparently does not. This relationship may be useful in the prediction of the release rate of various other $N_1$-alkoxycarbonyl 5-FU derivatives

Table 3
Release rate constants (α)

| Compound | α ($\mu g/\sqrt{t}$) | Receptor phase |
|---|---|---|
| I | 10.9 | pH 7.4[a] |
| II | 9.1 | pH 7.4[a] |
| III | 6.5 | pH 7.4[a] |
| III | 8.9 | 80% calf serum[c] |
| III | 8.5 | pH 10.8[b] |
| IV | 2.4 | pH 7.4[a] |
| IV | 1.8 | pH 10.8[b] |
| IV | 2.1 | 80% calf serum[c] |
| V | 0.8 | pH 7.4[a] |
| V | 0.9 | 80% calf serum[c] |
| VI | 0.65 | pH 7.4[a] |
| VII | 0.58 | pH 7.4[a] |

[a]0.02 M phosphate buffer ($\mu = 0.5$).
[b]0.02 M carbonate buffer ($\mu = 0.5$).
[c]80% calf serum in 0.02 M phosphate buffer at pH 7.4.

with the aim of selecting derivatives possessing a clinically appropriate release rate. In conclusion, this study indicates that the in vitro release rate of 5-FU from the prepared drug delivery systems can be controlled in a predictive manner by the lipophilicity of the prodrugs. The drug delivery system may have potential in the treatment of PVR but further in vivo toxicity investigations as well as determination of the in vivo release rates of 5-FU need to be done.

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