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# GENERALIA

*Editorial remarks.* There is still an urgent medical need for effective and welltolerated drugs for the treatment of the most common forms of cancer, such as bronchial carcinoma, or for post-operative prophylaxis against relapse and metastasis. - The old-established screening method based on rapidly proliferating acute transplantable lymphatic leukemias in the mouse that is applied in the major cancer research centers has certainly achieved some measure of clinical success, inasmuch as the mean duration of survival of patients with acute lymphatic leukemia has increased from 3 months to about 6 years and similar activity has been found in some rapidly proliferating lymphomas, sarcomas and teratomas.
The authors were convinced, however, that chemotherapeutic agents effective against lung cancer could only be found with the help of new specific animal models. They developed a model of an autochthonous tumor in the hamster, applied it in extensive series of experiments, and succeeded in synthesizing and identifying a group of compounds that were both effective and well tolerated. They describe the synthesis and biological activity of CGP 15720, the compound with the highest therapeutic index and an apparently non-cytotoxic mode of action.

## Ureido-ethyl-imidazolines active against autochthonous diethylnitrosamine-induced epidermoid, papillary and adenocarcinomatous tumors of the respiratory tract of Syrian hamsters and against human bronchogenic carcinomas in nu/nu mice

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*Summary.* The detection of a new class of tumor inhibiting substances is described. Employing a chemical reaction discovered several years ago, a series of imidazolinylureas were prepared. It was found that some compounds of this group were active against diethylnitrosamine (DENA)-induced tumours in hamsters. CGP 15720 A (1-{2-[2-(4-pyridyl)-2-imidazoline-1-yl]-ethyl}-3-(4-carboxy-phenyl)urea, **Xb**), the most active compound at present, was developed through a series of structural variations. CGP 15720 A inhibits significantly in oral or parenteral treatment with well tolerated doses (10-30 mg/kg) the progressive growth of autochthonous, DENA-induced papillary, epidermoid and adenocarcinomatous tumors of the respiratory system in Syrian hamsters and prolongs significantly the survival. The substance also inhibits significantly the growth of 2 poorly differentiated human epidermoid or anaplastic bronchogenic carcinomas in nu/nu Balb/c mice and prolongs the mean survival time. In these mice, the substance is also active against the rodent ascites tumors Ehrlich carcinoma, CrSa 180 and Yoshida Sa AH 66, although it is only marginally active or inactive against these tumors in normal mice or rats. - In the therapeutic trials, hamsters tolerated the highest dose administered for 4 weeks, 1000 mg/kg p.o., without signs or symptoms of toxicity.

### *1. Introduction*

In 1957, one of us published a method for preparing 1-amino-ethyl-substituted imidazolines from nitriles and diethylene triamine[1]. Application of this reaction to a large number of arylnitriles (**I**, scheme 1), aryloxyacetonitriles (**IIIa**), arylaminoacetonitriles (**IIIb**) and arylacetonitriles (**IIIc**) led to imidazolines with an aminoethyl side chain of types **II** and **IVa-c**. These 1-aminoethyl and also the corresponding 1-dimethylaminoethyl imidazolines were for the most part devoid of any characteristic biological activity; this did not hold true for the bis-imidazolines (**V** and **VI**) achieved by way of an analogous reaction. The reaction of 1 mole of triethylene tetramine and 2 moles of one of the above nitriles (**I** or **III**) with catalytic amounts of hydrogen sulphide or carbon disulphide resulted in 1,1'-ethylene-bis-(2-aryl-imidazolines) (**V**) or 1,1'-ethylene-bis-[2-(aryl-X-$CH_2$)-imidazolines] (**VIa-c**) possessing some interesting properties. 1,1'-ethylene-bis-[2-(4-ditert.-octyl-phenoxymethyl)-imidazoline] (**VII**), for instance, displayed inhibitory ef-

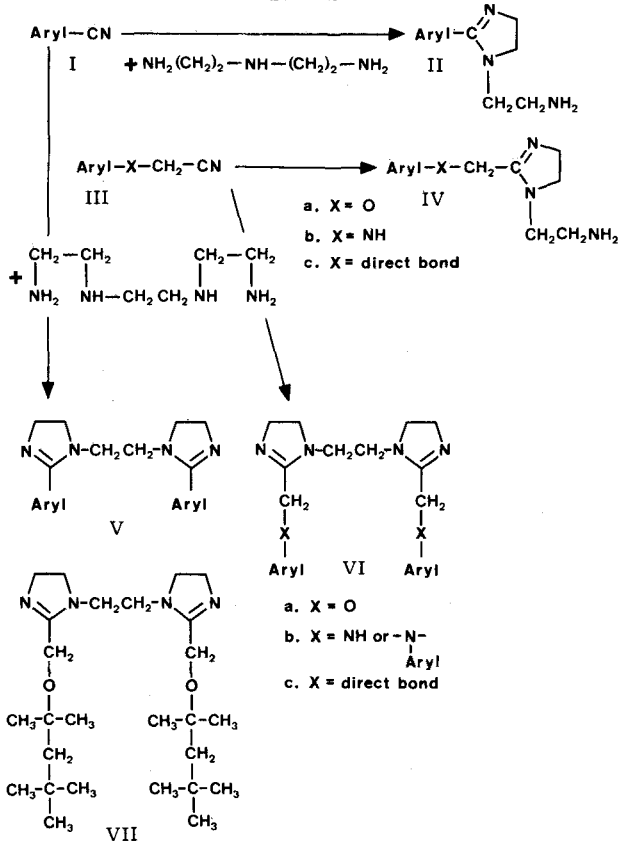
fects on transplantable tumors. Clinical evaluation of this compound was, however, precluded by its intense irritancy upon s.c. and i.v. injection. Other representatives of type **VI** showed antibacterial activity.

These findings prompted us to investigate the effects of novel imidazolines of both types on animal tumors. Dichloroanilinoacetonitrile served as the starting point for a series of 1-aminoethyl-2-(2,6-dichloroanilinomethyl)-imidazolines **VIII** (scheme 2); but our initial compounds in this series, e.g. the free amine **VIIIa** and its N,N-dimethyl derivative **VIIIb,** proved ineffective. These failures bore out a working hypothesis we had formulated earlier: namely that compounds posessing 2 strongly basic centers are rarely likely to display pharmacological activity. Apart from the curarizing compounds, the ganglion blockers, and certain antiprotozoal substances, there are very few biologically active dibasic amines: in all other branches of pharmacology, compounds with 1 basic center are superior. Proceeding on this assumption, we therefore tried 'closing' the strongly basic side-chain in **VIII,** first with the aid of a urea group. By way of the virtually inactive N'-methyl-urea **VIIIc** and its thio-analogue **VIIId,** we arrived at 1-{2-[2-(2,6-dichloro-anilinomethyl)-2-imidazolinyl-1]-ethyl}-3-(p-tolyl)-urea **(VIIIe),** the first substance to display marked inhibitory activity against DENA-induced tumors of the respiratory tract. Attempts to improve this activity by structural modification eventually led, after the inevitable to-and-fro between the chemical ideas and biological activities, to a new series of imidazolines, **IX,** starting from a o-chlorbenzonitrile. Here again, as in series **VIII,** the N,N'-dimethyl **IXb** and the N-methyl-ureido-derivatives **IXc** proved inactive, so that from then on we confined ourselves to aromatically substituted ureas. Our subsequent efforts were rewarded by 1-{2-[2-(2-chlorophenyl)-2-imidazolinyl-1]-ethyl}-3-(p-tolyl)-urea, **(IXe),** which displayed optimal activity against the above-mentioned tumors of the respiratory tract. Toxicity studies in mice and rats showed the compound to be well tolerated; this, however, was not the case in the dog, where it provoked emetic effects. The chemical investigation was thus faced with the dual problem of finding equally active compounds and at the same time eliminating possible side effects.

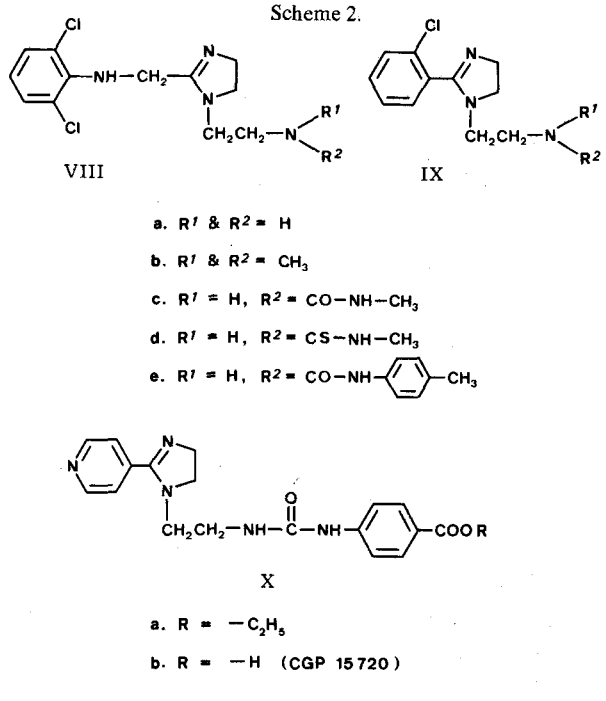
Although side effects are usually inseparably linked with the pharmacodynamic properties of the molecule, in this particular instance the problem was surmountable.

Many investigations of the metabolism of methyl groups on an aromatic ring have shown that these undergo oxidation in the body to carboxyl groups. The idea of replacing the p-tolyl group by a carboxyphenyl group to diminish the liposolubility of the substance proved to be the right approach since the emetic component disappeared completely. Additional replacement of the o-chlorophenyl residue by the pyridin ring finally resulted in the well tolerated compound **Xb,** 1-{2-[2-(4-pyridyl)-2-imidazoline-1-yl]-ethyl}-3-(4-carboxyphenyl)-urea (CGP 15720 A), the antitumor activity of which is reported below. The

Scheme 1.




Scheme 2.



base of **Xb** has a melting point of 181–184 °C; it forms a monohydrochloride with a m.p. of 217 °C and a methane sulphonate with a m.p. of 203–205 °C. This new class of substances offered many possibilities for variations which we investigated thoroughly.

The discrepancy between the effects produced by potential antineoplastic agents in tests on animals with transplantable tumors and their clinical efficacy, especially in cases of carcinoma, can be very wide. This is well exemplified by the bis-ethyleneimino-hydroquinones[2–4], the diacylamino-bis-ethyleneimi-nobenzoquinones[5–7] and the bis-guanylhydrazones, which led to the much debated compound DDUG[8–11]. The disappointing clinical activity of the available cytostatic drugs against epidermoid, adenomatous or anaplastic lung carcinoma is well known, and it therefore seemed necessary to develop new models better able to reliably predict therapeutic efficacy in human bronchogenic carcinoma. In this respect, we believe that induced tumors of the respiratory tract of Syrian hamsters afford a reasonably valid therapeutic model of human bronchogenic epidermoid and adenomatous and laryngeal carcinoma[12–14]. CGP 15720 A is the most active compound against these tumors at present.

## 2. *Experimental section*

### 2.1. *Chemistry*

*1-Aminoethyl-2-(4-pyridyl)-2-imidazoline* (**II**, aryl = 4-pyridyl). A mixture of 52 g (0.5 mole) 4-cyano-pyridine and 56.8 g (0.55 moles) diethylenetriamine and 1 ml carbondisulfide are gradually warmed to 110 °C, whereupon an exothermic reaction starts bringing the temperature to 125 °C without external heating. When this reaction is over the mixture is kept at 110 °C for 4 h. It is then distilled at 0.015 torr, boiling point 130–132 °C. The distillate crystallizes, melting point 44–47 °C. Yield 78%.

*1-{2-[2-(4-Pyridyl)-2-imidazoline-1-yl]-ethyl}-3-(4-ethoxy-carbonyl-phenyl)-urea* (**Xa**). 34 g (0.179 mole) of the above compound are suspended in 150 ml toluene, 34.1 g (0.179 mole) ethyl-4-isocyanato-ben-zoate is dropped in at room temperature and the mixture is stirred for 3 h at 90 °C. The oil, which separates, crystallizes upon cooling, raw melting point about 130 °C (61 g). It is purified by preparing the dihydrochloride in acetonic solution with alcoholic hydrochloride acid. Yellow crystals, melting point 178–180 °C.

*1-{2-(4-Pyridyl)-2-imidazoline-1-yl]-ethyl}-3-(4-carbo-xy-phenyl)-urea* (**Xb**). 68.1 g (0.179 mole) of **Xa** base are dissolved in 1.3 l ethanol, 300 ml of aqueous 2N sodium hydroxyde is added and the mixture stirred for 15 h. The solution is completely neutralized by adding 0.6 mole of glacial acetic acid, and evaporated to about $\frac{1}{3}$ of its volume, whereupon **Xb** crystallizes on cooling. It is washed with cold water and isopropanol giving a melting point of 181–184 °C. The monohydrochloride prepared out of this base has a m.p. of 217 °C (dec.), while the methanesulfonate has a m.p. of 205 °C (dec.).

### 2.2. *Chemotherapy*

#### 2.2.1. *Material and methods*

In the studies with *Syrian hamsters,* the papillary, epidermoid and adenocarcinomatous tumors (figs 1–6) are induced in 50-day-old female hamsters (BIO

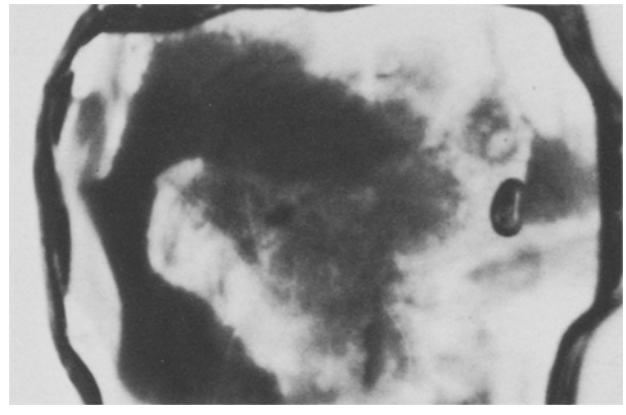


Figure 1. 'In toto' examination. Papillomatous-epidermoid tumor of the larynx. × 17.

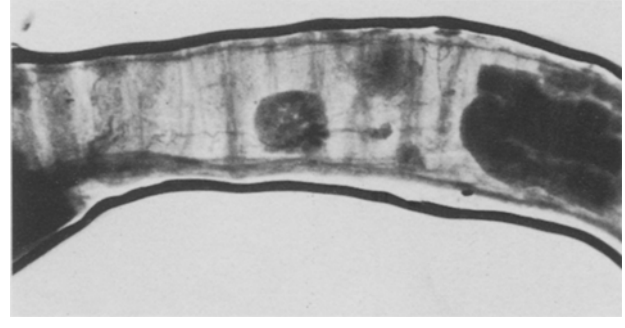


Figure 2. 'In toto' examination. 2 smaller papillomatous and 1 epidermoid tumor of the trachea. × 6.

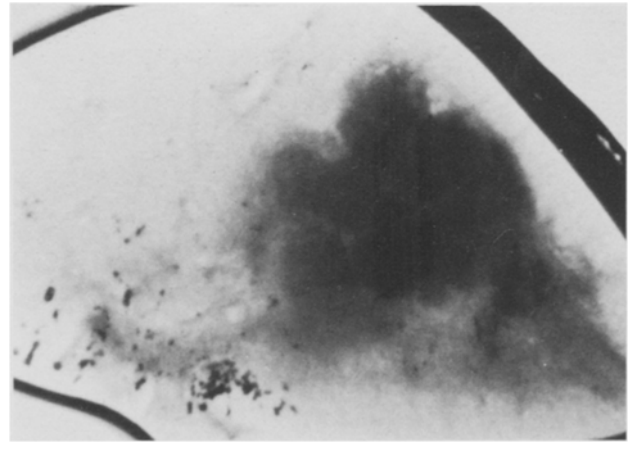


Figure 3. 'In toto' examination. Combined adenomatous-epider-moid carcinoma; left upper lobe of the lung. × 6.

8720, Telaco, Main USA, inbred; or Lak:LVG (Syr) outbred) by oral dosing with 10 mg/kg diethylnitrosamine (DENA) twice weekly for 10 weeks. 9 or 10 weeks later, the test compounds are given daily or intermittently for 4 weeks. At autopsy, all organs are examined macroscopically, and the larynx, trachea and lobes of the lung microscopically. The histological techniques we used initially revealed all of the tumors in the trachea; lung tumours, however, could only be quantitatively assessed in serial sections. We therefore devised a method permitting stereomicroscopic ('in toto') evaluation of all tumors in cleared preparations of the larynx, trachea and lungs[15]. These tissues were then examined histologically. The main criterion in evaluating the therapeutic activity of test substances is the mean percent reduction in the tumor area in relation to the placebo-treated controls. Under these conditions adriamycin, CCNU [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea], and bleomycin were investigated in comparison with CGP 15720 A. In survival experiments, treatment was also started 9 or 10 weeks after induction and continued until death.

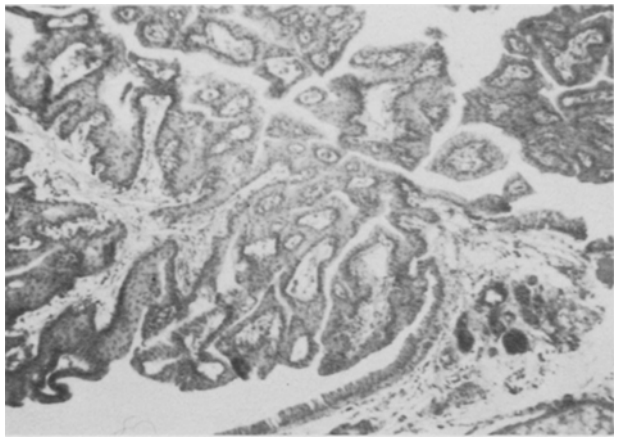


Figure 4. Histological examination. Papillomatous-epidermoid tumor of the larynx. ×40.

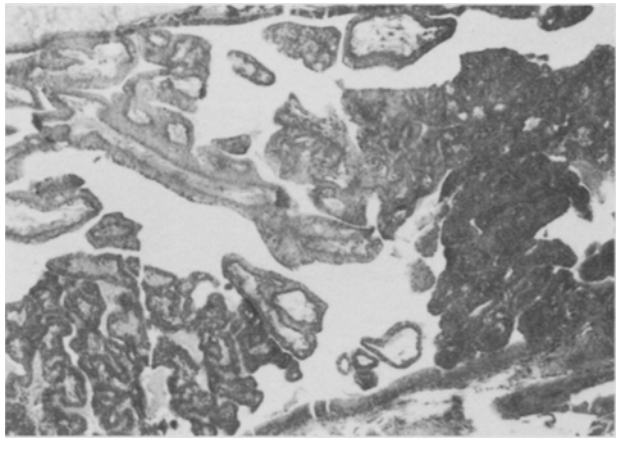


Figure 5. Histological examination. Papillomatous-epidermoid tumors of the trachea. ×40.

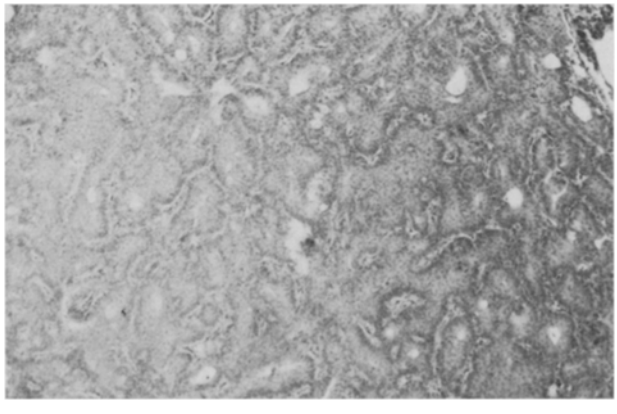


Figure 6. Histological examination. Combined adenomatous-epidermoid carcinoma in the left upper lobe. ×37.

Each dose of CGP 15720 A was given in a volume of 0.5 ml/100 g hamster of a solution of 0.5% CMC (Tylose C 600, Hoechst) and 20% 1.2-propylene glycol in distilled water; in this medium, 2% of the substance is dissolved at a pH 4.5.

In the studies with *nu/nu Balb/c mice* (Tif [SPF] or Bom, [SPF]) $10^6$ cells of ascites tumors of mice (Ehrlich carcinoma, CrSa 180) or rats (Yoshida sarcoma AH 66), obtained 7 days after transplantation, were injected i.p. in 0.2 ml. Pieces of approximately 20 mg of the poorly differentiated human bronchogenic carcinomas 'Hotz'/1908 and MBA/9812, propagated in nu/nu Balb/c mice, were transplanted s.c. with a trocar under light ether anesthesia. Treatment was started 2 or 3 days after transplantation (table 4). In 1 experiment with nu/nu Swiss mice, $5 \times 10^6$ cells from tissue culture were transplanted s.c. (fig. 12).

## *3. Results*

### 3.1. *Antitumor activity*

In *Syrian hamsters,* oral daily administration of CGP 15720 A (1000, 300, 100, 30 and 10 mg/kg) caused a marked and significant tumor reduction[16]. In each of 4 different trials in altogether 67 treated animals and

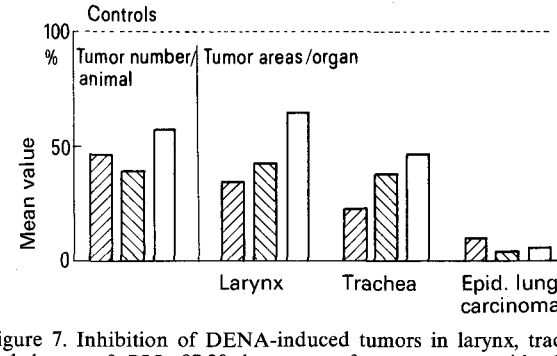



Figure 7. Inhibition of DENA-induced tumors in larynx, trachea and lungs of BIO 87.20 hamsters after treatment with CGP 15720 A; 'in toto' examination. ▨, 20×100 mg/kg p.o. daily, 5 times weekly. ▧, 20×30 mg/kg p.o. daily, 5 times weekly. □, 20×10 mg/kg p.o. daily, 5 times weekly. Mean of number and areas of tumor in percent of controls.

63 controls (T/C), daily treatment with 30 mg/kg p.o., less than $^1/_{100}$ of the maximum tolerated dose, was significantly and reproducibly effective (table 1a, figs 7 and 8); the T/C (treated/controls) ratio of the mean tumor areas was 2.3±0.9/6.7±2.0 mm². The difference of the degree of tumor reduction in effective doses was not statistically significant within the dose-range 10 mg/kg to 1000 mg/kg, although a maximum of activity seemed to be observed with 300 mg/kg p.o. 3 administrations daily of 100 mg/kg or 300 mg/kg p.o. were not more active than 1 daily administration. Peritoneal administration of 100, 30 and 10 mg/kg was also significantly active but the degree of activity was not different from that when the same doses were given p.o. (table 1a).

In comparison to CGP 15720 A, adriamycin, bleomycin and CCNU proved to be less active. Adriamycin (0.4 mg/kg i.p., intermittently) and CCNU (6.25 mg/kg p.o., intermittently) reduced tumor area by 49% and 46%, respectively. Daily treatment with 0.01 mg/kg adriamycin i.p. in a total dose of 0.2 mg/kg had the same effect as intermittent high-dose therapy with a total of 3.2 mg/kg i.p.; bleomycin (0.2 mg/kg i.p., intermittently) led to only marginal reduction (table 1b). Higher doses of these agents were not more effective and provoked toxic symptoms. Cyclophosphamide methotrexate and 5-fluorouracil, which have so far only been evaluated by histological methods, reduced the tracheal tumors by only 30–40%, even at doses close to the limit of tolerability: 15 mg/kg s.c., 0.5 mg/kg i.p. and 12.5 mg/kg s.c. daily, respectively (not shown).

Combined treatment with CGP 15720 A (20×30 mg/kg p.o.) and bleomycin (8×0.2 mg/kg p.o., twice weekly) or CCNU (20×2.5 mg/kg p.o.) produced at least an additive effect; the combined action of CGP 15720 A and adriamycin was less impressive (table 1b).

CGP 15720 A also reduced the number and areas of adenocarcinomata, which are less common than epidermoid or papillary tumors. The relative frequency of these was only 6% in 105 treated as against 34% in 50 control animals; the mean area of the tumors was only 0.02 mm² as compared with 0.44 mm² in the controls (not shown).

All foregoing results gleaned from the 'in toto' examination were almost entirely confirmed by histological examination.

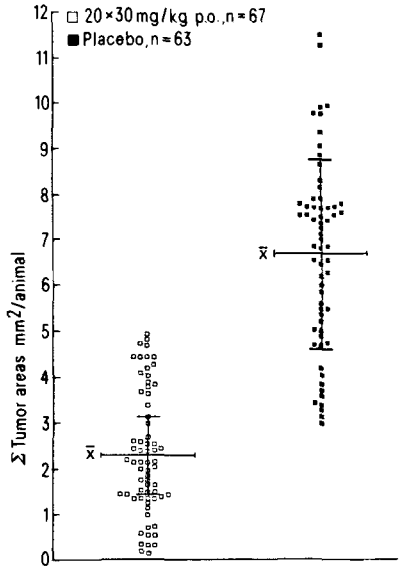



Figure 8. Inhibition of DENA-induced tumors in larynx, trachea and lungs of BIO 87.20 hamsters after treatment with CGP 15720 A; 'in toto' examination of 4 experiments.

Table 1a. Effect of CGP 15720 A, adriamycin, bleomycin and CCNU in Syrian hamsters with DENA-induced tumours of the respiratory tract

| Substance | Dose (mg/kg) | No. of animals | Tumor reduction[a] Area | Number |
|---|---|---|---|---|
| CGP 15720 A | 20× 1000 p.o.[b] | 16 | −65* | −47 |
| | 20× 300 p.o. | 18 | −83* | −60 |
| | 20× 100 p.o. | 18 | −76* | −47 |
| | 20× 30 p.o. | 67[c] | −66* | −45 |
| | 20× 10 p.o. | 16 | −54* | −42 |
| | 20× 3 p.o. | 14 | −40 | −36 |
| | 20× 1 p.o. | 14 | −30 | −36 |
| | 20× 0,3 p.o. | 14 | −20 | −20 |
| | 60× 300 p.o.[d] | 20 | −75* | −57 |
| | 60× 100 p.o. | 19 | −73* | −50 |
| | 20× 100 i.p. | 15 | −78* | −50 |
| | 20× 30 i.p. | 11 | −49* | −49 |
| | 20× 10 i.p. | 15 | −50* | −27 |
| | 20× 10 i.p. | 21 | −60* | −50 |
| | 20× 3 i.p. | 16 | −45* | −42 |
| | 20× 1 i.p. | 19 | −35 | −10 |
| | 20× 0.3 i.p. | 19 | −25 | − 0 |

[a] Mean percent reduction in relation to controls; 'in toto' examination. [b] Daily, 5 times weekly. [c] 4 different trials. [d] 3 times daily. [e] 4 consecutive daily doses, weeks 1 and 4. [f] Twice weekly. *$2\alpha \leq 0.01$ (Wilcoxon rank test). **Significantly more active than CGP 15720 A alone.

Table 1b. Effect of CGP 15720 A, adriamycin, bleomycin and CCNU in Syrian hamsters with DENA-induced tumors of the respiratory tract

| Substance | Dose (mg/kg) | No. of animals | Tumor reduction[a] Area | Number |
|---|---|---|---|---|
| Adriamycin | 8×0.4 i.p.[e] | 19 | −49* | −27 |
| | 20×0.01 i.p. | 18 | −47* | −40 |
| CGP 15720 A + adriamycin | 20× 30 p.o. 20×0.01 i.p. | 18 | −82* | −63 |
| Bleomycin | 8×0.2 i.p.[f] | 17 | − 8 | − 4 |
| | 20×0.08 i.p. | 18 | −17 | − 3 |
| CGP 15720 A + bleomycin | 20× 30 p.o. 8×0.2 i.p. | 18 | −90** | −55 |
| CCNU | 8×6.25 p.o.[f] | 20 | −46* | −19 |
| | 20×2.5 p.o. | 18 | − 6 | −17 |
| CGP 15720 A + CCNU | 20× 30 p.o. 20×2.5 p.o. | 15 | −95** | −66 |

Legend: see table 1a.

In *survival experiments,* in which CGP 15720 A was administered in a dose of 30 mg/kg p.o. daily for 4 weeks, then 5 times weekly until death, the survival of the treated animals (n=50) was significantly prolonged[16]. Treatment of Syrian hamsters with CGP 15720 A (30 mg/kg p.o.) prolonged the mean survival time more than did a polychemotherapeutic regimen (12.3±5.8 as against 8.7±4.9 weeks, Figure 9). Bleomycin alone (0.08 mg/kg i.p.) was marginally active (9.2±5.0 weeks: control 7.0±3.5 weeks, fig. 10). Concomitant treatment with CGP 15720 A (30 mg/kg p.o.) and bleomycin (0.08 mg/kg i.p.) produced a more pronounced prolongation of the mean survival time (16.7±8.5 weeks: control 7.0±3.5 weeks, fig. 10) than did treatment with CGP 15720 A alone (12.6±5.8 weeks: control 6.5±3.6 weeks) in 2 previous experiments with 90 treated and 3 with 138 controls (fig. 11).

In *studies with nu/nu mice,* when given in 20 doses of 100 mg/kg p.o. from the 2nd or 3rd day after transplantation onwards, the compound significantly inhibited the growth of the poorly differentiated human bronchogenic epidermoid carcinoma 'Hotz'/1908 and prolonged significantly ($2p < 0.001$, Student's t-test) the mean survival time (table 2, fig. 12), and inhibited strongly the anaplastic carcinoma MBA/9812 in oral as well as peritoneal administration of a sterile, neutral solution (tables 3 and 4). Although it had no or only marginal effect on some common transplantable tumors on normal host (Ehrlich ascites carcinoma, CrSa 180/ascites in Balb/c-, Lewis lung carcinoma in C57 Bl-, leukemia L1210/ascites in $B_6$ $D_2$ $F_1$-mice, Yoshida AH/66/ascites and Walker CaSa 256/solid in Wistar rats), it inhibited for instance Ehrlich

Table 2. Inhibition of the human undifferentiated bronchogenic epidermoid carcinoma 'Hotz' in syngeneic female nu/nu Balb/c mice by oral treatment with CGP 15720 A

| Animal | Dosage 20× 100 mg/kg p.o. 5 times weekly Tumor weight (g) | Control, placebo[a] |
|---|---|---|
| 1 | 0.05 | 0.23 |
| 2 | 0.10 | 0.22 |
| 3 | 0.07 | 0.26 |
| 4 | 0.07 | 0.23 |
| 5 | 0.11 | 0.24 |
| 6 | 0.06 | 0.26 |
| 7 | 0.01 | 0.19 |
| 8 | 0.10 | 0.18 |
| 9 | 0.02 | 0.19 |
| 10 | 0.09 | 0.26 |
| $\bar{X}$±SD | 0.068±0.034 | 0.226±0.031 |
| $\Delta$ % | −69.9[b] | |

[a] Solution of CMC (Tylose C600 Hoechst) 0.5% and 1,2-propylene glycol 20% in distilled water. [b] $2p \leq 0.001$ Student's t-test.

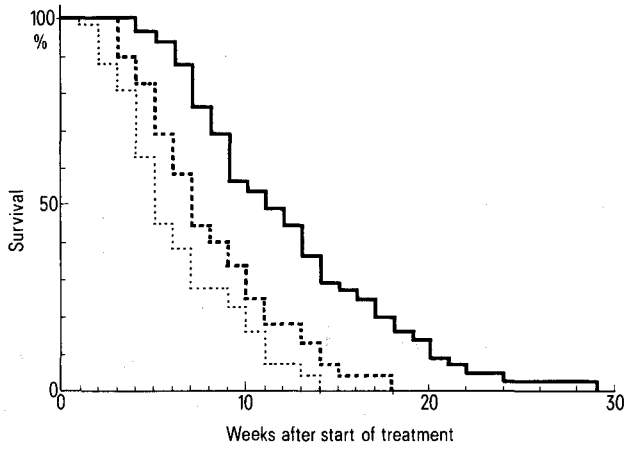



Figure 9. Effect of CGP 15720 A on survival time in female Lak:LVG (Syr) hamsters with DENA-induced tumors of the respiratory tract in comparison with a clinical combination. n=45/group.

| | | | | |
|---|---|---|---|---|
| —, CGP 15720 A | 30. | mg/kg p.o. | 5 times weekly | |
| ----, Adriamycin | 0.5 | mg/kg i.p. | 4 times weekly | (weeks 1, 4, 7) |
| Oncovin | 0.04 | mg/kg i.p. | 1 time weekly | (weeks 1, 4, 7) |
| Cytoxan | 1.5 | mg/kg p.o. | 5 times weekly | |
| Metho-trexate | 0.4 | mg/kg i.p. | 1 time weekly | (weeks 1, 3, 5, 7, 9) |
| Fluoro-uracil | 5.0 | mg/kg i.m. | 1 time weekly | (weeks 2, 4, 6, 8) |

····, Control (placebo)

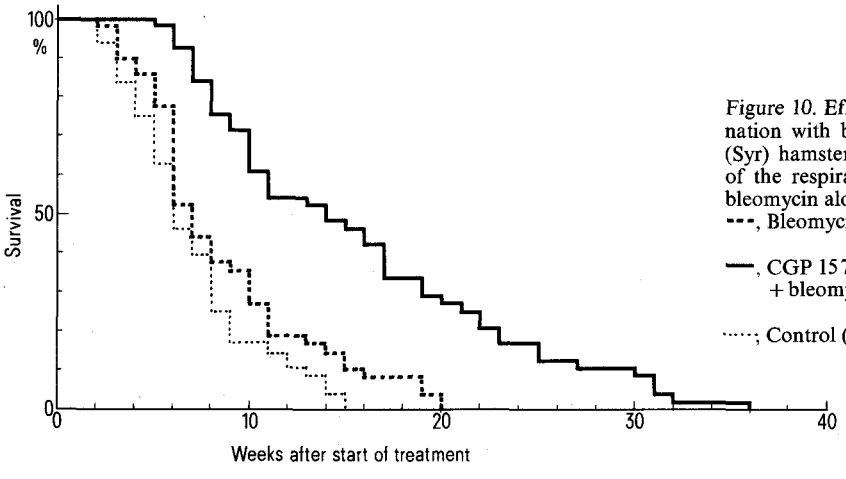



Figure 10. Effect of CGP 15720 A in combination with bleomycin in female Lak:LVG (Syr) hamsters with DENA-induced tumors of the respiratory tract in comparison with bleomycin alone. n=48/group.

| | |
|---|---|
| ---, Bleomycin | 0.08 mg/kg i.p. 5 times weekly until death |
| —, CGP 15720 A + bleomycin | 30 mg/kg p.o. 0.08 mg/kg i.p. 5 times weekly until death |

·····, Control (placebo)

ascites carcinoma, CrSa 180 and Yoshida ascites hepatoma AH66 in nu/nu Balb/c mice (table 5, fig. 13). The mechanism of this interesting phenomenon is being investigated. The activity of cyclophosphamide against these tumors in nu/nu mice (table 5) was comparable to that observed in normal mice in many previous experiments (standard compound).

### 3.2. *Tolerability*

CGP 15720 A is well tolerated. In female mice and hamsters, given a single dose and then observed for 7 days, the $LD_{50}$ was approximately 5000 mg/kg p.o. In the therapeutic trials, hamsters tolerated the highest dose administered for 4 weeks, 1000 mg/kg p.o., without signs or symptoms of toxicity. The white-blood cell (WBC) counts were increased by 46% and 36% after treatment of female hamsters with $20 \times 1000$ and 300 mg/kg p.o., respectively (initial WBC 11899: final WBC 17310 and 16168). In a 30-day toxicity study in Sprague-Dawley rats and Beagle dogs, the highest daily doses, 250 mg/kg i.p. and p.o. and

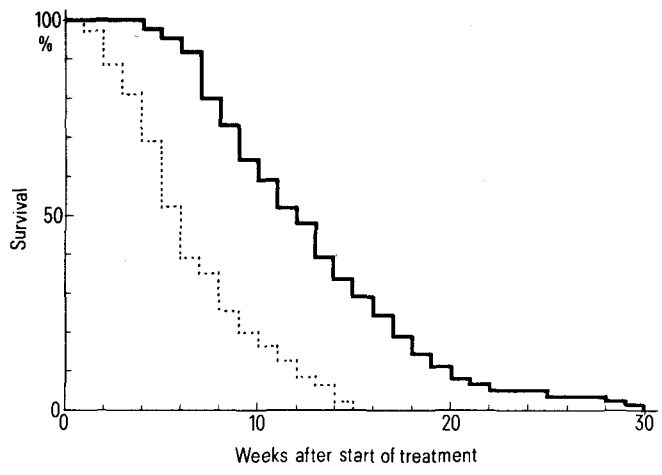



Figure 11. Effect of CGP 15720 A on survival time in female Lak:LVG (Syr) hamsters with DENA-induced tumors of the respiratory tract. —, CGP 15720 A 30 mg/kg p.o. 5 times weekly, n = 90 (2 experiments); ·····, controls (placebo), n = 138 (3 experiments).

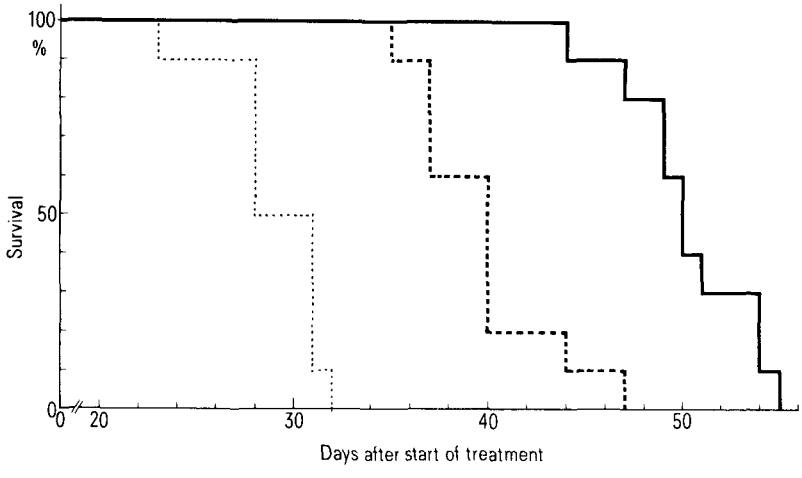



Figure 12. Effect of CGP 15720 A on survival time in female Swiss nu/nu mice with the human bronchogenic epidermoid carcinoma 'Hotz'. n = 10/group. —, $20 \times 100$ mg/kg p.o. 5 times weekly; ---, $20 \times 30$ mg/kg p.o. 5 times weekly; ·····, control (placebo).

Table 3. Inhibition of the human undifferentiated bronchogenic carcinoma MBA 9812 in syngeneic female nu/nu Balb/c mice by oral treatment with CGP 15720 A

| Animal | Dosage $15 \times 100$ mg/kg p.o. 5 times weekly | Dosage $15 \times 30$ mg/kg p.o. 5 times weekly | Dosage $15 \times 10$ mg/kg p.o. 5 times weekly | Control, placebo[a] |
|---|---|---|---|---|
| | Tumor weight (g) | | | |
| 1 | 0.006 | 0.026 | 0.114 | 0.692 |
| 2 | 0.014 | 0.069 | 0.342 | 0.584 |
| 3 | 0 | 0.082 | 0.092 | 0.888 |
| 4 | 0.036 | 0.196 | 0.133 | 0.788 |
| 5 | 0.019 | 0.199 | 0.115 | 0.621 |
| 6 | 0.006 | 0.202 | 0.320 | 0.555 |
| 7 | 0 | 0.187 | 0.176 | 0.407 |
| 8 | 0.006 | 0.036 | 0.344 | 0.932 |
| 9 | 0 | 0.057 | 0.094 | 0.591 |
| 10 | 0.036 | 0.061 | 0.107 | 0.698 |
| $\bar{x} \pm SD$ | 0.012±0.014 | 0.112±0.074 | 0.184±0.107 | 0.676±0.160 |
| $\Delta$ % | −98.2[b] | −83.4[b] | −72.8[b] | |

[a] Solution of CMC (Tylose C 600 Hoechst) 0.5% and 1,2 -propylene glycol 20% in distilled water. [b] $2p \leq 0.001$ Student's t-test (mean percent reduction in relation to controls).

500 mg/kg p.o., respectively, were well tolerated without gross or histological symptoms; no evidence of toxicity or mutagenicity was found in rat embryos after administration of 150 mg/kg s.c. from days 8 to 13 of pregnancy[17,18].

### *4. Discussion*

The poor response to monotherapy with cytostatic drugs in inoperable bronchogenic carcinoma[19] was found to be improved by certain combinations[20,21]; even so, the overall median survival time, for instance, was only 47.5 weeks in patients with non-small-cell carcinoma treated with a combination of cis-dichlorodiamine platinum **(II)**, adriamycin, cyclophosphamide, CCNU and vincristine[22] or about 11 months in patients with small-cell carcinoma after alternating courses of polychemotherapy with cyclophosphamide and methotrexate, or CCNU, vincristine, adriamycin and procarbazine or VP-16-213 and ifosfamide, given for 6 weeks each[23]. As post-operative and adjuvant therapy, another cytostatic combination (cyclophosphamide, methotrexate and vinblastine) prolonged

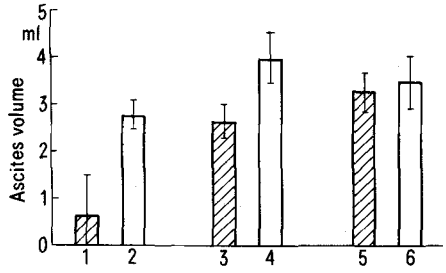



Figure 13. Inhibition of Ehrlich ascites carcinoma by treatment with CGP 15720 A in nu/nu Balb/c, syngeneic Balb/c and NMRI mice. n = 10/group. *1* and *2* nu/nu Balb/c female mice, ▨, 100 mg/kg p.o., □, placebo. *3* and *4* syngeneic Balb/c female mice, ▨, 100 mg/kg p.o., □, placebo. *5* and *6* NMRI female mice, ▨, 125 mg/kg p.o., □, placebo.

Table 4. Inhibition of human undifferentiated bronchogenic carcinoma MBA 9812 transplanted s.c. in syngeneic female nu/nu Balb/c mice by peritoneal and oral treatment with 15720 A

| Substance | Solution in | Dosage mg/kg[a] 5 days weekly | No. of animals | Tumor reduction in percent[b,c] |
|---|---|---|---|---|
| Sterile CGP 15720 A | $NaHCO_3$[d] | 15× 100 i.p. | 9 | −87.4* |
| | $NaHCO_3$[d] | 15× 30 i.p. | 8 | −84.2* |
| | $NaHCO_3$[d] | 15× 10 i.p. | 9 | −78.4* |
| | $NaHCO_3$[d] | 15× 10 i.p.[e] | 8 | −70.8* |
| | $NaHCO_3$[d] | 15× 3 i.p. | 8 | −73.9* |
| | $NaHCO_3$[d] | 15× 3 i.p.[e] | 8 | −55.5* |
| | $NaHCO_3$[d] | 15× 1.0 i.p.[e] | 8 | −47.8 |
| | $NaHCO_3$[d] | 15× 0.3 i.p.[e] | 8 | −41.0 |
| | $NaHCO_3$[d] | 15× 10 p.o. | 9 | −68.7* |
| | $NaHCO_3$[d] | 15× 3 p.o. | 9 | −58.8* |
| Normal CGP 15720 A | CMC/PG[f] | 15× 10 i.p. | 8 | −74.4* |
| | CMC/PG[f] | 15× 3 i.p. | 8 | −71.9* |
| | CMC/PG[f] | 15× 10 p.o. | 9 | −69.5* |
| | CMC/PG[f] | 15× 3 p.o. | 9 | −58.1* |
| | CMC/PG[f] | 15× 1 p.o.[e] | 8 | −44.0 |
| | CMC/PG[f] | 15× 0.3 p.o.[e] | 8 | − 0 |
| Controls (untreated) | | | 9 | |

[a] Start of treatment 3 days after transplantation. [b] 19 days after transplantation. [c] Mean percent reduction in relation to controls. [d] 1.4% sterile neutral $NaHCO_3$ solution. [e] 2nd experiment. [f] CMC (Tylose C 600 Hoechst) 0.5% and 1.2-propylene glycol 20% in distilled water. * $2p \leq 0.001$. Student's t-test.

Table 5. Effect of CGP 15720 A and cyclophosphamide against Ehrlich ascites carcinoma, Yoshida AH66 and CrSa 180 sarcoma in syngeneic female nu/nu Balb/c mice

| Dose mg/kg daily for 4 days[a] | Effect[b] Ehrlich ascites ml | % reduction[c] | Yoshida ascites ml | % reduction | CrSa 180 ascites ml | % reduction |
|---|---|---|---|---|---|---|
| CGP 15720 A 100 p.o. | 1.82 | −64.2** | 0.19 | −93,4** | 3.73 | −36.7** |
| 30 p.o. | 3.04 | −40.3** | 1.77 | −38.8** | 4.44 | −24.6** |
| 10 p.o. | 3.82 | −25.0** | 2.37 | −18.0* | 3.96 | −32.8** |
| 100 i.p. | 0.08 | −98.4** | 0.02 | −99.3** | 2.03 | −65.5** |
| 30 i.p. | 0.06 | −98.8** | 0.14 | −95.2** | 3.59 | −39.0** |
| 10 i.p. | 3.71 | −27.1** | 2.51 | −13.1** | 3.56 | −39.6** |
| Cyclophosphamide 50 i.p. | 0.08 | −98.4** | 0.04 | −98.6 | 0.01 | −99.8** |
| Control | 5.09 | | 2.89 | | 5.89 | |

[a] 1st administration 4 h after transplantation. [b] Examination 10 days after transplantation. [c] % tumor reduction in relation to controls. * $p < 0.05$ Student's t-test. ** $p < 0.01$ Student's t-test.

the survival of about 30% of patients with the faster-growing oat cell carcinoma, but no beneficial effect was seen in patients with squamous-cell carcinoma and adenocarcinoma[24].

Combinations of this kind are thus obviously much less effective in the treatment of bronchogenic carcinoma than on the treatment of acute lymphoblastic leukemia in children[25,26] or some rapidly proliferating lymphomas, sarcomas and teratomas; this may be a reflection of the fact that under experimental conditions they are predominantly active against rapidly proliferating tumors, e.g. lymphatic leukemias and some other transplantable tumors of mice. To detect substances or combinations capable of inhibiting the slower and more irregular growth of human primary bronchial or other carcinomata, however, other experimental models showing similar growth kinetics[27] would be more appropriate. It seems reasonable to assume that any therapy effective against solid autochthonous, chemically induced or spontaneous tumors, might also be more successful in primary human tumors[28-35].

In the particular case of human bronchogenic carcinoma, induced tumors of the respiratory tract of the Syrian hamster appear to afford a highly predictive therapeutic model of human bronchogenic carcinoma. If a compound shows significant activity in this model - as does CGP 15720 A - there is a greater likelihood of its being effective in human non-small cell carcinoma. As regards CGP 15720 A, this assumption is strengthened by the fact that it is also active against non-small cell human bronchogenic carcinomata in nu/nu mice. The discrepancy between its being active against some transplantable tumors in these mice on the one hand, and yet, being inactive against the same tumors in normal mice could indicate that thymus-deprived suppressor cells or a thymus factor may antagonize an immunological mechanism of action of the substance. This latter mechanism is hypothesized. The activities and the good tolerability suggest that CGP 15720 A is probably a useful drug for treatment of epidermoid, anaplastic and adenomatous bronchogenic carcinoma and larynx carcinoma.

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