Therapy of Human Transitional Cell Carcinoma of the Bladder by Oral Administration of the Epidermal Growth Factor Receptor Protein Tyrosine Kinase Inhibitor 4,5-Dianilinophthalimide

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ABSTRACT

Epidermal growth factor receptor (EGF-R), a transmembrane glycoprotein that mediates the mitogenic response of cells to epidermal growth factor, is highly expressed on malignant human bladder cancer cells. The 4,5-dianilinophthalimides represent a novel class of inhibitors of the EGF-R family of tyrosine kinase with selectivity at the enzymatic and cellular levels. Two compounds of this class, CGP 54211 and CGP 53353, inhibited tyrosine kinase activity of the EGF-R in five different human transitional cell carcinoma lines. The compounds also produced cytostasis in vitro. Highly metastatic human 253J B-V cells were implanted in the bladder wall of nude mice. The daily oral administration of CGP 54211 inhibited the level of EGF-R phosphorylation in this tumor; necrosis and inhibition of tumor growth paralleled this inhibition.

INTRODUCTION

EGF-R, a transmembrane glycoprotein, mediates the mitogenic response of TCC of the bladder to EGF and TGF-α (1, 2). The urine is a rich source of EGF (3), and the expression level of EGF-R by TCC cells correlates directly with proliferation (4, 5) and, hence, stage and grade of the disease (3, 6). The EGF-R consists of an external binding domain, a transmembrane region, and an intracellular domain with PTK activity (1, 2, 7). Following ligand binding, the PTK initiates a signal that culminates in mitosis (7). Theoretically, the inhibition of EGF-R PTK activity should inhibit EGF-stimulated DNA synthesis. Although several classes of PTK inhibitors have been identified, most synthetic compounds exhibited limited selectivity for the cellular EGF-R PTK and limited in vivo efficacy (8-14).

The purpose of this study was to determine whether specific inhibition of PTK activity of the EGF-R by 4,5-dianilinophthalimides (CGP 54211 and CGP 53353), which are ATP-competitive protein kinase inhibitors (15, 16), could produce therapeutic benefits against human TCC of the bladder. We report that these compounds inhibited PTK activity of the EGF-R and produced cytostasis in vitro of highly metastatic human 253J B-V cells. The oral administration of the PTK inhibitor CGP 54211 also inhibited growth of human bladder cancer cells implanted orthotopically in nude mice.

MATERIALS AND METHODS

Tumor Cell Cultures. The human TCC cell lines 253J B-V, 5637, TCC, T-24, and RT4 were grown as monolayer cultures in EMEM supplemented with 5% fetal bovine serum, vitamins, sodium pyruvate, L-glutamine, and nonessential amino acids. The supplemented medium was free of endotoxin, as determined by the limulus amebocyte lysate assay (Associates of Cape Cod, Woods Hole, MA). The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air. All cultures were free of mycoplasma, reovirus type 3, pneumonia virus in mice, K virus, encephalitis virus, lymphocyte choriomeningitis virus, ec-tromelia virus, and lactate dehydrogenase virus.

Reagents. The 4,5-dianilinophthalimides CGP 52411 and CGP 53353 were gifts of Ciba-Geigy, Ltd. (Basel, Switzerland). Both compounds were dissolved in 10 mM DMSO for the in vitro experiments and in 100% ethanol for the in vivo studies. Genistein, purchased from Sigma Chemical Company (St. Louis, MO), was dissolved in 10 mM DMSO. Monoclonal antiphosphotyrosine antibody (4G10) and rHuEGF were purchased from United Biochemical, Inc. (Lake Success, NY). Mouse anti-EGF-R antibody was purchased from Oncogene Science, Inc. (Uniondale, NY). Sheep anti-EGF-R polyclonal antibody was purchased from United Biochemical, Inc.. Mouse anti-activated EGF-R Mabs were purchased from Chemicon International, Inc. (Temecula, CA) and Zymed Laboratories, Inc. (San Francisco, CA).

Flow Cytometric Analysis for Expression of EGF-R. The expression of EGF-R by the different human TCC lines was determined by flow cytometry. Single-cell suspensions were fixed in 70% ethanol and incubated with the mouse antihuman EGF-R antibody at a 1:100 dilution and then resuspended with a second FITC-conjugated goat antimouse antibody. Tumor cells incubated only with the second antibody served as a control. The cells were then resuspended in culture medium at
5 x 10^6 cells/ml and analyzed by an Elite flow cytometer (Coulter Corp., Hialeah, FL). The cell-bound FITC EGF-R fluorescence was computer analyzed, and the expression of EGF-R was calculated as relative fluorescence units normalized with the control secondary antibody fluorescence profile. All five cell lines expressed the EGF-R by fluorescence-activated cell sorting analysis (Fig. 1).

**Determination of TP by Western Blotting.** Human TCC cells growing in culture were plated into six-well plates at a cell density ranging from 5-10 x 10^5 cells/800 mm² well overnight in 5% EMEM. The cells were then rinsed with HBSS, incubated in serum-free medium for 4 h, and treated with genistein, CGP 54211, or CGP 53353 at concentrations of 1, 10, 30, or 100 μM. Treatment with 1% DMSO was served as a control, because this was the highest concentration of DMSO used to dissolve the 100 μM concentration of inhibitors. The cells were then stimulated with 20 ng/ml rHuEGF for 10 min, washed five times with PBS containing 1 mM sodium orthovanadate (Na3VO4) and 5 mM EDTA, and scapped into 0.2 ml of lysis buffer [1% Triton X-100, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1 mM Na3VO4, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 20 μM leupeptin, and 0.15 units/ml aprotinin]. The lysate was placed on ice for 20 min and then centrifuged at 12,000 rpm for 15 min at 4°C. Triton X-100-soluble protein was saved; diluted to 2 mg/ml in 62.5 mM Tris-HCl (pH 6.8), 2.3% SDS, 100 mM DTT, and 0.005% bromphenol blue; and boiled. The proteins (15–30 μg) were resolved on 10% SDS-PAGE and transferred onto 0.45-μm nitrocellulose membranes. After the filters were blocked with 3% BSA/1% ovalbumin in TBS [20 mM Tris-HCl (pH 7.5) and 150 mM NaCl], they were probed overnight with tyrosine-specific Mab 4G10 (0.2 μg/ml) in TTBS (TBS and 0.1% Tween 20) at 4°C and then washed three times in TTBS. The filters were incubated for 1 h with 1:2000 horseradish peroxidase-conjugate Fab(’2) goat antimouse antibody (Amersham Corp., Arlington Heights, IL) and rinsed as above. As a control, blots were stripped and reprobed with sheep anti-human EGF-R polyclonal antibodies (United Biochemical, Inc.), and detected using a horse radish peroxidase-labeled, secondary anti-sheep antibody (1:2000; Amersham Corp.; Ref. 17). All blots were detected with an enhanced chemiluminescence system (Amersham Corp.).

**Antiproliferative Effects of PTK Inhibitors.** Tumor cell cytostasis was determined using the MTT assay. Briefly, viable tumor cells in the exponential growth phase were plated in 96-well microtiter plates at a concentration of 2000 cells/well in 10% EMEM. Twenty-four h later, the cells were refed with medium (control) or either CGP 54211, CGP 53353, or genistein at a concentration between 0 and 100 μM. Ninety-six h later, the cells were incubated with medium containing MTT at 0.42 mg/ml for 2 h. The cells were then lysed with DMSO. The conversion of MTT to formazan by metabolically viable cells was measured by a microplate scanning spectrophotometer at 57 nm (Dynatech, Inc., Chantilly, VA). Cytostasis was calculated according to the formula:

\[
\text{Cytostasis (\%) = 1 - \frac{A}{B} \times 100}
\]

where A is the absorbance of the treated cells, and B is the absorbance of the control cells.

**Animals.** Male athymic BALB/c nude mice were obtained from the Animal Production Area of the National Cancer Institute Frederick Cancer Research Facility (Frederick, MD), housed in laminar flow cabinets under specific pathogen-free conditions, and used at 8–12 weeks of age. Animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture, Department of Health and Human Services, and National Institutes of Health.

**Orthotopic Implantation of Cells.** Cultured 253J B-V cells (Ref. 18; 50% confluence) were given fresh medium 24 h before harvest. The cultures were then rinsed in Ca²⁺- and Mg²⁺-free HBSS and overlaid for 2 min with a 0.25% trypsin-0.02% EDTA solution. The flask was tapped to dislodge the cells, which were then pipetted gently to produce a single-cell suspension. The cells were washed in HBSS and their viability ascertained by trypan blue exclusion. The cell suspension was kept at 4°C. Only single-cell suspensions with >95% viability were used for the in vivo studies.

Mice anesthetized with methoxyflurane were placed in the supine position. The abdomen was cleaned with iodine and alcohol. A lower midline abdominal incision was made, and the bladder was exteriorized. Tumor cells (1 x 10⁶) were injected into the dome of the bladder using a 30-gauge needle and a 1-ml disposable syringe (injection volumes ranged from 0.05 to 0.1 ml). A well-localized bleb was the sign of a technically satisfactory injection. The abdominal incision was closed in one layer using metal clips (18).

**Therapy of Human TCC Growing in the Bladder Walls of Nude Mice.** To evaluate the therapy of established tumors, oral administration of compound CGP 54211 began on day 7 following tumor implantation. This compound was used because its TK inhibition was more selective for EGF-R TK than was CGP 53353 in vitro (16). Mice received oral therapy with 25 mg/kg of CGP 54211 5 days a week for 4 weeks; it was dissolved in 100%
ethanol containing 1 drop of Tween 80 per ml and stirred until a clear solution was obtained. This dose was well tolerated and resulted in significant in vivo tumor growth inhibition in previously reported studies (16). Control animals received oral ethanol.

In a separate series of experiments, mice were treated with oral genistein to evaluate its effect on the therapy of established tumors. Therapy began 7 days following tumor implantation. Mice received oral therapy with 50 μg of genistein dissolved in DMSO at dilutions of 1:100 and 1:500, 5 days a week for 4 weeks.

Necropsy Procedures. Mice were killed by cervical dislocation on day 36, and the presence of local bladder cancer and metastasis was evaluated. No evidence of metastasis was observed at the time of necropsy. The bladders were weighed and processed for histology or for protein extraction.

Immunohistochemical Analyses. 253J B-V cells growing in culture were starved overnight in 0% serum-containing medium, treated with 40 ng/ml EGF for 15 min in the presence or absence of CGP 54211 (30 μM), fixed in cold acetone for 10 min, treated 12 min with 3% hydrogen peroxide in methanol (v/v), and then incubated in protein-blocking solution [5% normal human serum/0.5% normal goat serum in PBS (v/v)] for 15 min. The cells were immunolabeled at 4°C in a humidified chamber with a mouse monoclonal (Mab) anti-activated EGF-R clone Z026 (1:50; Zymed Laboratories, Inc., San Francisco, CA), which detects only the activated (tyrosine phosphorylated form) of EGF-R; it does not cross-react with other phosphorylated proteins or inactivated EGF-R (19, 25). The samples were rinsed with PBS, incubated in protein-blocking solution for 60 min. The samples were treated with the chromogen substrate 3,3′-diaminobenzidine, rinsed, and mounted in Permount before photography.

At necropsy, the tumor tissue from animals treated with vehicle alone or with CGP 54211, or positive control tumor tissue of A431 epidermoid carcinoma growing in the subcutis of nude mice was cut into 5-mm pieces, placed in OCT compound (Miles Laboratories, Elkhart, IN) in 1-inch aluminum caps, and snap frozen in liquid nitrogen. Sections (8–10 μm) were picked up on slides (Fisher Scientific, Pittsburgh, PA) and air dried for 30 min. The slides were fixed in cold acetone for 10 min, treated 12 min with 3% hydrogen peroxide in methanol (v/v), and then incubated in protein-blocking solution [5% normal human serum/0.5% normal goat serum in PBS (v/v)] for 15 min. Two different primary antibodies were used for analysis of tissue sections: (a) a mouse anti-activated EGF-R Mab (Chemicon International, Inc., Temecula, CA), which reacts strongly and specifically with the activated and phosphorylated human EGF-R and does not react with other phosphorylated proteins (20); and (b) a sheep antihuman EGF-R polyclonal antibody (United Biochemical, Inc.) that does not distinguish between activated and inactivated EGF-R. The sections were incubated with the primary antibody in a humidified chamber for 15–18 h at 4°C. The sections were rinsed and incubated with the protein-blocking solution for 10 min before incubating with the appropriate peroxidase-conjugated secondary antibody (1:200 [v/v]) for 1 h at ambient temperature. Positive reaction was visualized by incubating the slides with stable 3,3′-diaminobenzidine (Research Genetics, Huntsville, AL) for 10–20 min. The sections were rinsed with distilled water and counterstained with Gill’s hematoxylin for 1 min. The sections were dried and mounted in Permount and examined in a bright-field microscope. Images were digitized using a Sony 3CCD color video camera (Sony Corp. of America, Montvale, NJ) and a personal computer equipped with an Optimas Image Analysis Software (Optimas Corporation, Bothell, WA). Composite images were printed on a Sony dye sublimation printer.

Statistical Analysis. The results were analyzed by the Wilcoxon χ² test and the Student’s t test (two-tailed).

RESULTS

Inhibition of EGF-stimulated Protein TP by CGP 54211 and CGP 53353. In the first set of experiments, we determined whether the TK inhibitors CGP 54211 and CGP 53353 inhibited EGF-stimulated TP in human TCC cells. Stimulation of 253J B-V cells with 20 ng/ml rHuEGF for 10 min induced the TP of three proteins with apparent molecular masses of 170, 51, and 39 kDa as detected using antiphosphotyrosine antisera (Fig. 2A). The 170-kDa band was identified as EGF-R by Western blot analysis using anti-EGF-R antibodies (see below). TP was inhibited by CGP 54211 and CGP 53353 in a dose-dependent manner but was not affected by genistein, even at concentrations of up to 100 μM (Fig. 2A).

In the next set of experiments, we extended the study to four other human TCC lines, TCC, RT4, T-24, and 5637. The data shown in Fig. 2B demonstrate that stimulation of all five human TCC cells with 20 ng/ml rHuEGF induced the specific TP of the EGF-R, which was inhibited by the presence of 30 μM CGP 54211 or CGP 53353, but not by the PTK inhibitor genistein. The TK inhibitors did not affect the in vitro expression of the EGF-R, as shown by reprobing the identical blot with anti-EGF-R-specific antibodies (Fig. 2B; see below). These studies also confirm the 170-kDa protein band as the EGF-R and not p185 c-erbB-2, and that the decrease in TP in cells treated with CGP 54211 and CGP 53353 was not due to unequal protein loading. These experiments confirm previous observations that these two compounds preferentially suppress EGR-R tyrosine kinase phosphorylation at these concentrations (15, 16).

Antiproliferative Effect of TK Inhibitors on Human TCC Cells in Vitro. In the next set of studies, we determined whether the incubation of five different human TCC cell lines with CGP 54211 or CGP 53353 would inhibit cell proliferation in vitro. At concentrations ranging from 1 to 10 μM, the TK inhibitors produced significant cytostasis. A representative study using the 253J B-V cells is shown in Fig. 3. The cytostasis was reversible, and once the drugs were washed off, tumor cell growth resumed (data not shown). At the concentrations used here, the inhibition by both CGP 54211 and CGP 53353 is relatively specific for the EGF-R kinase (15, 16), although a nonspecific toxic effect cannot be completely ruled out. We also evaluated the antiproliferative effects of both CGP54211 and CGP53353 against the SW620 human colon carcinoma cells, which do not express the EGF-R. No cytostasis was observed at concentrations up to 100 μM compared with DMSO vehicle alone (data not shown).

In Vivo Therapy of Orthotopic 253J B-V Tumors with CGP 54211. Seven days after the implantation of 253J B-V cells into the wall of the bladder, groups of nude mice were randomized to receive oral treatments with ethanol (control) or CGP 54211. Treatment was administered five times a week for
A

PTK Inhibitors (µM)

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B

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Fig. 2 Inhibition of EGF-induced protein TP by CGP 54211 and CGP 53353. A, TP induced in 253J B-V cells by 20 ng/ml EGF for 10 min was inhibited by CGP 54211 and CGP 53353 in a dose-dependent manner but not by genistein. CGP 54211 and CGP 53353 were used at 1, 3, 10, 30, and 100 µM, and genistein was used at 1, 10, and 100 µM. B, treatment of the human TCC lines 253J B-V, 5637, TCC, RT4, and T24 with 20 ng/ml EGF for 10 min induced TP. No change in EGF-R levels was noted following treatment with either drug or shown by reprobing of the blot in B with an anti-EGF-R antibody demonstrating similar protein loading between lanes. Cells were either pretreated with EGF (+) or untreated (−). TP was inhibited by treatment with either 30 µM CGP 54211 or CGP 53353, respectively.

4 weeks (20 oral treatments). No cumulative toxicity was observed in mice receiving 25 mg/kg/day of CGP 54211. Tumors were present in the bladders of 10 of 10 ethanol-treated mice and in 9 of 10 mice receiving CGP 54211 (Table 1). However, tumor weights differed significantly between the two groups. In control mice, the median bladder weight was 172 mg (range, 52–384), whereas in mice receiving CGP 54211 it was 62 mg (range, 23–186; P < 0.005). Moreover, upon histological examination, significant areas of necrosis were found within all the tumors of the CGP 54211-treated mice. The tumors growing in control mice were free of necrotic areas (Fig. 4). This indicates that therapy with CGP 54211 resulted in tumor destruction, not just growth inhibition, and that tumor growth inhibition was more substantial than that indicated only by tumor weight. This in vivo experiment was repeated with similar results (data not shown). Therapy with genistein was well tolerated at the doses used. However, no inhibition of tumor growth was observed in mice treated with genistein versus those treated with DMSO alone (Table 2).

Immunohistochemical Analysis of EGF-R and Activated EGF-R in 253J B-V Cells Growing in Culture or in the Bladders of Nude Mice following Therapy with CGP 54211. The EGF-R content and the level of activated EGF-R (tyrosine-phosphorylated form) was analyzed in 253J B-V cells growing in culture and in situ in the bladders of mice following therapy with CGP 54211. Two different and specific Mabs were used, each of which recognizes only the activated or tyrosine-phosphorylated form of EGF-R (19, 20, 25), as well as a polyclonal antibody that does not distinguish activated from inactivated EGF-R (see "Materials and Methods"). 253J B-V cells treated with EGF in culture showed enhanced immunostaining with the anti-activated EGF-R Mab compared with control untreated cells or cells treated with both EGF and CGP 54211 (Fig. 5, compare C with B and D); these experiments correlate directly with the Western blot analyses demonstrating increased EGF-R IF following stimulation with EGF and inhibition by CGP 54211 (Fig. 2). Similarly, histochemical staining for activated EGF-R in 253J B-V tumors growing in the bladders of mice demonstrated specific immunoreactivity in tumors from control vehicle alone-treated mice, whereas immunoreactivity was undetectable in CGP 54211-treated tumors (Fig. 6, compare F with G). In contrast, analysis of the identical tumors for total EGF-R
showed similar levels of immunoreactivity in CGP 54211-treated and untreated specimens (Fig. 6, compare B and C). Sections of A431 squamous cell carcinoma growing in the subcutis of nude mice, known not only to overexpress EGF-R but also to exhibit a high degree of EGF-R autophosphorylation, similarly demonstrated positivity (Fig. 6, compare A and E). These data indicate a reduction of activated EGF-Rs in 253J B-V tumors treated with CGP 54211 compared to controls without a concomitant decrease in total EGF-R levels, suggesting an inhibition of EGF-R function and not a reduction in protein levels.

**DISCUSSION**

Human TCC overexpresses the EGF-R relative to normal urothelium, and the urine is a rich source of EGF. Increased expression of EGF-R by human TCC cells is associated with a poor prognosis (3–6). The EGF-R mediates the proliferative effects of EGF and TGF-α by activation of a TK (1, 2). Several potentially useful methods of interfering with activation of EGF-R are available. Anti-EGF-R Mabs that block ligand binding and thus interfere with EGF-stimulated TK activity have been shown to inhibit the *in vitro* growth of tumor cells stimulated with EGF or TGF-α, as well as the growth of human squamous cell carcinoma xenografts bearing high levels of EGF-R (8, 9). Anti-EGF-R antibodies have also been conjugated to toxins and cytotoxic drugs with the intention of increasing their antitumor activity (21). Preclinical studies showed that a panel of human TCC lines is sensitive *in vitro* to the one such chimeric molecule, TP-40 (22), that can be administered by intravesical instillation. Such a chimeric molecule may not be

![Fig. 3](image-url)  
*Fig. 3* Antiproliferative effect of CGP 54211 and CGP 53353 against 253J B-V. Significant cytostasis of 253J B-V was achieved at doses between 1 and 10 μM of both CGP 54211 and CGP 53353. Cytostasis achieved by genistein is shown. A similar antiproliferative effect against the other four cell lines was observed.

![Fig. 4](image-url)  
*Fig. 4* Representative appearance of 253J B-V tumor growing within the bladder of athymic nude mouse from (A) untreated control, showing solid tumor formation, and (B) a mouse receiving CGP 54211, showing extensive necrosis within the tumor mass. Magnification, ×150.

### Table 1  
*In vivo* therapy of 253J B-V with CGP 54211

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<td>62</td>
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* One representative experiment of three.

One representative experiment of three.

Control vehicle alone consisted of 100% ethanol with 1 drop of Tween 80/ml (see "Materials and Methods").

### Table 2  
*In vivo* therapy of 253J B-V with genistein

<table>
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* One representative experiment of three.

Control alone consisted of HBSS.

Difference between 1:500 dilution of DMSO and genistein was not significant.

Difference between 1:100 dilution of DMSO and genistein was not significant.
able to penetrate the urothelium to reach tumor cells bearing the EGF-R, which may account for the results of a recent clinical study, in which TP-40 produced only low levels of antiproliferative effects on human bladder carcinomas (23).

A number of vastly different compounds can inhibit TK activity. In the present study, we demonstrated both selective inhibition of EGF-R TK phosphorylation and resultant antiproliferative effect against a panel of human TCC lines that expresses the EGF-R by a novel class of agents, the dianilinophthalimides (15, 16). Additionally, minimal nonspecific cytostasis of SW620 cells (which do not express the EGF-R) was observed following in vitro exposure to these compounds (data not shown). The oral administration of the drug CGP 54211 was well tolerated by athymic nude mice when given at the dose of 25 mg/day, 5 days a week for 4 weeks. This dose significantly inhibited the growth of the 253J B-V tumors grow-

Fig. 5 Immunohistochemical staining of 253J B-V cells growing in culture with a mouse anti-activated EGF-R antibody. A, A431 epidermoid carcinoma cells (positive control); B, untreated 253J B-V cells; C, 253J B-V cells treated with EGF; D, 253J B-V cells treated with EGF + CGP 54211; E, 253J B-V cells treated with CGP 54211 alone; F, negative control for immunohistochemistry (secondary antibody alone) of untreated 253J B-V cells.
Fig. 6 Immunohistochemical staining of 253J B-V tumors growing within the bladder of athymic nude mice with sheep polyclonal anti-EGF-R antibody (A–D) or a mouse anti-activated EGF-R Mab (E–H). A and E, s.c. tumor section of A431 epidermoid carcinoma growing in nude mice (positive control; Ref. 25); B and F, tumor section from control untreated mice, showing immunoreactivity specific for total EGF-R (B) or the activated EGF-R (tyrosine autophosphorylated form; F); C and G, tumor section from mice receiving CGP 54211, showing no change in total EGF-R immunoreactivity (C) but undetectable immunostaining with mouse anti-EGF-R (activated) Mab (G); D and H, negative control (secondary antibody alone)-treated tumor sections.
ing orthotopically in the bladders of nude mice. Similar therapeutic results using CGP 54211 have been observed against both A431 and SK-OV-3 xenografts (16). It is significant to note that these compounds had no in vivo antiproliferative effect against v-sis-transformed BALB/c 3T3 cells, which proliferate in response to platelet-derived growth factor (15, 16).

Therapy in vivo was accompanied by a significant reduction in activated EGF-R and not total EGF-R protein as indicated by immunohistochemical analyses. These results confirm the inhibitory effect of this drug on EGF-R autophosphorylation (i.e., function) in vivo. In similar experiments, in vitro incubation of 253J B-V cells with CGP 54211 also inhibited EGF-R autophosphorylation and not total protein levels as determined by Western blot and immunohistochemical analyses. Collectively, these studies demonstrate a reduction in activated EGF-Rs following treatment in vitro and in vivo with CGP 54211, suggesting inhibition of EGF-R function and hence growth inhibition.

Dianilinophthalimide represents a novel class of competitive TK inhibitors with selectivity for the EGF-R at the enzymatic, cellular, and in vivo levels (15, 16). These compounds are related structurally to the potent but nonselective staurosporine and its aglycon and diindolylmaleimide derivatives (15). However, because of its three-dimensional steric conformation, the dianilinophthalimides are more selective competitors of the EGF-R TK. These data therefore suggest that these compounds and others (24) that selectively inhibit EGF-R TK phosphorylation hold promise as therapeutic modalities for bladder and other cancers, such as metastatic human colon carcinoma, that overexpress the EGF-R (17).

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Therapy of human transitional cell carcinoma of the bladder by oral administration of the epidermal growth factor receptor protein tyrosine kinase inhibitor 4,5-dianilinophthalimide.

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