PROCEEDINGS

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(Arranged by Subject Category)

with longer treatment duration (Adler, et al., 1994; Au et al., 1998). The aim of this study is to compare the cytotoxicity of different exposure times of ET-743, a new marine natural product with promising antitumor activities, to assist clinical studies in finding a rational schedule for Phase II trials. Eighteen different types of tumor cells, three of them derived from pediatric tumors, and two non malignant cells were used and exposed to concentrations of ET-743 ranging from 0.0001 to 0.1 μg/mg. For drug treatments and activity, a modification was used of the method described by Bergeron et al., 1984. To determine whether the higher activity observed for the longer treatments is due to a delayed exhibition of drug's effects and/or a reflection of cumulative effects that require a continuous drug exposure, cells were treated with ET-743 for 1, 3, 6, 8 & 24 hours and then either: (1) immediately processed for drug effect measurement (cell loss or immediate effect); or seeded again in drug-free medium, cultured and processed for drug effect measurement at 72 hours (cell inhibition or delayed effect). Data obtained and statistical analysis have shown that immediate and delayed effects have different pharmacodynamics. The immediate effect is higher with increasing exposure times up to 24 hours. For delayed effect, although short times exposures presented lower IC_{50}'s, the gain of sensitivity is more apparent between 3 and 8 hours.

#1996 Bisubstituted tricyclic chromophores as small molecule inhibitors of human telomerase: Biological and modelling studies. [1]Kelland, L.R., [1]Gowan, S.M., [2]Harrison, R.J., [2]Wood, A.A., [2]Read, M.A., [2]Dosanjh, H.S., [2]Perry, P.J., [2]Reszka, A.P., [2]Davies, R.T., and [2]Neidle, S. *[1]CRC Centre for Cancer Therapeutics and [2]CRC Biomolecular Structure Unit, Inst. of Cancer Research, Sutton, SM2 5NG, UK.*

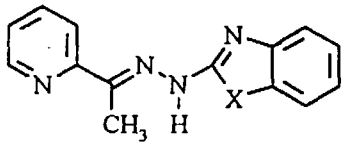
Telomerase may provide a novel tumor-selective target for anticancer drug design. Our telomerase inhibitory strategy is aimed at the design of small molecules capable of stabilising G-quadruplexes and thereby preventing the requirement of telomerase for a non-folded telomere DNA primer. A biological test cascade has been established where compounds are sought which possess a wide therapeutic index between potent cell-free telomerase inhibition (and no *Taq* polymerase inhibition) and low acute cytotoxicity against tumor cells. We initially observed activity with disubstituted amidoanthraquinones (AQs). We now show that potent activity is not specific to Aqs as other tricyclic compounds (acridine and fluorenones) inhibited human telomerase in an *in vitro* cell-free PCR-based assay (e.g., 50% inhibition at concentrations ranging from <1 to >50 μM). Parallel growth inhibition studies (96 h drug exposure, sulforhodamine B assay) have shown almost all compounds to be markedly less cytotoxic than doxorubicin or mitoxantrone (mean IC_{50} of approx 10 nM and 0.5 nM respectively) with IC_{50} values typically in the 0.5 to 25 μM range. Molecular modelling studies, performed on a human four-telomere intramolecular folded structure, have now determined structures and energetics for ligand complexes. They have provided rationales for the observed structure-activity relationships, and a basis for structure-aided drug design. Lead molecules are undergoing further *in vitro* (detection of telomere erosion in tumor cells) and *in vivo* evaluation for antitumor effects.

#1997 Anti-tumor activity of prodrugs of triapine™, an inhibitor of ribonucleotide reductase. Li, Z., Luo, X., Chen, S.-H., Li, X.-Y., Li, J., Niu, C., Karra, S., Wang, Q., Barrows, S., Mao, J., Doyle, T., King, I. and Zheng, L.-M. *Vion Pharmaceuticals, Inc. New Haven, CT.*

Triapine™ (3-amino-4-methylpyridine-2-carboxaldehyde thiosemicarbazone), an inhibitor of ribonucleotide reductase, has good activities against a variety of experimental tumor models. To maximize its utility and to produce prodrugs with different rate of activation, a series of water-soluble phosphate prodrugs of Triapine™ was made. Two of the phosphate prodrugs, I (para—phosphate) and II (orthophosphate), have been thoroughly evaluated. The water solubility of prodrug I and II were 16 mg/ml and 11 mg/ml, respectively, whereas the parental compound was <1mg/ml. Triapine™ was readily detectable when the prodrugs were incubated with alkaline phosphatase. Using the murine M109 lung carcinoma model, we found prodrug II was more efficacious than prodrug I and the parental compound. Optimal anticancer activity of Triapine™ required a twice-daily dosing schedule whereas prodrug II exhibited good antitumor activity with a once-a-day dosing schedule. Triapine™ prodrug II was also active against murine B16 melanoma and the activity was compared to some of the anti-cancer agents currently used in clinics.

#1998 Benzothiazolyl, benzoxazolyl, and benzimidazolyl hydrazones derived from 2-acylpyridines: Synthesis and antitumor evaluation. Easmon, J., Heinisch, G., Pürstinger, G., Margreiter, E., Hofmann, J. *Institute of Pharmaceutical Chemistry, Innrain 52a, (J.E., G.H., G.P.), Institute of Medical Chemistry and Biochemistry, Fritz-Pregl-Strasse 3 (E.M., J.H.), University of Innsbruck, A-6020 Innsbruck, Austria.*

In search of inhibitors of ribonucleotide reductase, we considered it of high interest to replace the thiocarbamoyl [$CS(NH_2)$] function of 2-acetylpyridine thiosemicarbazone with a benzothiazole ring (compound **1**). Compound 1 and its congeners exhibited high cytotoxic activity *in vitro* against a panel of human tumor cell lines (Easmon, J., *et al. Eur. J. Med. Chem. (1997), 32, 397–408*). The bioisosteric replacement of the benzothiazole moiety by a benzoxazole or a benzimidazole ring resulted in compounds **2/3** which turned out to be more potent than **1** by a factor of 30. In an extension to these studies, compounds where the acetyl-CH_3 was replaced by various alkyl, cycloalkyl, phenyl and pyridine moieties were synthesized. The novel 2-acylpyridine hydrazones inhibited the proliferation of several human tumor cells (IC_{50} = 0.052–6.98 μM), induces apoptosis in Burkitt's lymphoma cells, and also are potent inhibitors of RNA synthesis. Financial assistance provided by the Austrian Science Foundation project no. P 12384-MOB.



1: X = S (IC_{50} = 0.15 - 5.31 μM)
2: X = O (IC_{50} = 0.006 - 0.23 μM)
3: X = NH (IC_{50} = 0.005 - 0.68 μM)

#1999 Pharmacological studies on two new classes of heterocyclic anticancer drug. Truong-Chiott, S.A., Haydar, S.N., Krapcho, P.A., and Hacker, M.P. *University of Vermont, Burlington, VT 05405.*

Aza-benzothiopyranoindazoles and aza-benzothiopyranopyridines, recently developed and synthesized in our laboratory, are two novel classes of compounds with impressive anti-tumor activity. They are structurally related to the Anthrapyrazoles and the Anthracyclines, but contain a heterocyclic rather than a carbocyclic ring. In vitro cytotoxicity data obtained from testing against L1210, S180 and S180/A10 cells demonstrated excellent antitumor activity with little or no cross resistance in the MDR cell line S180/A10. Anti-tumor activity of compounds from both classes was exquisitely sensitive to the location of the nitrogen within the heterocyclic ring, with the 9-azas superior to that of the 7 or 8 or non-azas. Ethidium bromide displacement assays using salmon sperm DNA were performed on four compounds (a 9-aza and an 8-aza from each class) to detect possible DNA-drug interaction. All four compounds were able to displace ethidium bromide from DNA and caused decreases in fluorescent intensities, indicating the existence of DNA-drug interaction and possibly DNA intercalation. To confirm that intercalation occurred, ligation assays of supercoiled DNA pBR322 in the presence and absence of drugs were conducted. All four compounds interacted with DNA through intercalation. However, comparisions of results from cytotoxicity assays and ethidium bromide displacement assays suggested that DNA interaction may not be the primary mechanism for potency of our compounds. (Research was supported in part by a grant from Boehringer Mannheim Italia, Milan Italy).

#2000 The effect of CCI-779, a novel macrolide anti-tumor agent, on the growth of human tumor cells *in vitro* and in nude mouse xenografts *in vivo*. Gibbons, J.J., Discafani, C., Peterson, R., Hernandez, R., Skotnicki, J., and Frost, P. *Oncology and Immunoinflammatory Research, Wyeth-Ayerst Research, Pearl River, NY 10965.*

CCI-779 is a sirolimus analog formulated for intravenous use. The growth of human tumor cells was either sensitive (IC_{50}~1nM) or relatively resistant (IC_{50}>1.0μM) when co-cultured *in vitro* with CCI-779. Growth inhibitory effects were blocked by the FKBP inhibitory molecule ascomycin, suggesting that the mechanism of action of CCI-779 is similar to sirolimus. Growth inhibited cells were arrested in the G1 phase of the cell cycle. PDGF stimulation of the human glioblastoma line T98G was markedly inhibited (IC_{50}~1pM) by CCI-779 in serum free medium. *In vivo* in nude mouse xenografts, the growth of staged human glioblastoma (U87MG) tumors was blocked by a variety of dosing regimens (minimum effective dose 0.1–1.0 mg/kg). Staged tumors treated for 5 consecutive days with CCI-779 were still growth inhibited 14 days later. In contrast, the effect of the compound on immune function was lost as early as 1 day after drug withdrawal. Various histological tumor types (pancreas, breast, prostate) were also sensitive to CCI-779 in nude mouse xenografts. The data suggests that CCI-779 will be an effective anti-tumor agent for several human tumor types when given via an intermittent dosing regimen. The compound currently is in Phase I trials in man.

#2001 Highly potent anthracycline-based antitumor agents. Priebe, W., Przewloka, T., Fokt, I., Ling, Y-H., Perez-Soler, R. *The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.*

New anthracyline-based agents designed to interact and crosslink with DNA in a base specific process have been synthesized. These analogs contain unique three ring system which is relatively stable. Synthesized compounds displayed activity significantly higher than that of parental daunorubicin or doxorubicin. In brief, in vitro the compound WP836 derived from doxorubicin was 500- to more than 100,000-fold more potent than doxorubicin in test performed in sensitive and multidrug resistant cell lines. Similarly, the increased activity was also noticed for analog WP809 obtained from daunorubicin. Other analogs were also designed and synthesized. Observed activity and high potency indicate that the primary mechanism of action of these analogs is different from doxorubicin and daunorubicin.