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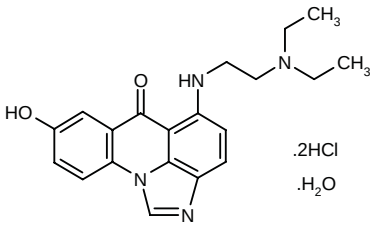
CCC: 0377-8282/98

C-1311

Antineoplastic

NSC-645809

5-[2-(Diethylamino)ethylamino]-8-hydroxy-6*H*-imidazo[4,5,1-*de*]acridin-6-one dihydrochloride hydrate



$C_{20}H_{22}N_4O_2.2HCl.H_2O$; Mol wt: 441.36

CAS: 138154-39-9 (as anhydrous free base)
CAS: 138154-55-9 (anhydrous)

EN: 227463

Synthesis

The synthetic procedure was carried out according to Cholody *et al.* (1) (Scheme 1). The starting 1-chloro-7-hydroxy-4-nitro-9(10*H*)-acridinone (I) was synthesized by the method of Capps (2). The condensation of (I) with 2-(diethylamino)ethylamine (II) in DMF gave 1-[2-(diethylamino)ethylamino]-7-hydroxy-4-nitro-9(10*H*)-acridinone (III) in good yield and purity. The reduction of the nitro group of (III) with hydrazine hydrate and Raney Ni in THF yielded the amino intermediate (IV), extremely unstable due to its sensitivity to oxidation, which was immediately used in the next step as crude hydrochloride salt. The salt (IV) was refluxed with 95% formic acid giving the imidazoacridinone derivative. The final product was isolated directly from the reaction mixture as hydrochloride salt after acidification with concentrated HCl and recrystalized from a mixture of methanol/acetone.

Description

Pale yellow needles, m.p. 250-5 °C with decomposition; free base m.p. 239-42 °C.

Introduction

Imidazoacridinones are a new group of potent antitumor compounds developed as a result of studies on the mechanism of cytotoxic and antitumor action of aminoanthraquinones, including the antitumor drug mitoxantrone. It was shown that diaminoalkyl groups in the side chains of mitoxantrone were a prerequisite not only for biological activity of this drug, as was shown by others (3), but also for its ability to induce interstrand cross-links in DNA (4, 5). It was hypothesized that attachment of a diaminoalkyl pharmacophoric group to other DNA intercalators (*e.g.*, acridinones) would result in compounds with antitumor properties. Further modification of diaminoalkylacridinone resulted in development of several groups of antitumor acridinones (1, 6-8). Among them, the most potent were imidazoacridinones (1, 6, 9), which contain in their structure a planar polycyclic acridinone moiety which intercalates to DNA, and two pharmacophoric groups which potentially could be involved in the covalent binding and cross-linking of DNA following metabolic activation. One of these groups is the diaminoalkyl side chain at position 5, which was necessary for DNA cross-linking by mitoxantrone (4, 5). Another one is the potential quinone-imine group formed by the hydroxyl group in position 8 and the heterocyclic nitrogen atom in the acridinone nucleus. This group could also be involved in covalent binding to DNA, which occurs after metabolic activation. The imidazole ring combines both pharmacophoric groups by two nitrogen atoms. The first nitrogen atom is a part of the potential quinone-imine structure, whereas the second one is placed at the position *para* to the diaminoalkyl side chain. The presence of such a *para* substituent was shown to be crucial for antitumor activity of several groups of acridinone derivatives with a diaminoalkyl side chain (8).

The most active derivative from the imidazoacridone group, C-1311, was selected and is currently undergoing phase I clinical trials. Pharmacological properties of this compound, as well as preliminary studies on the mechanism of its cytotoxic and antitumor activities are presented below.

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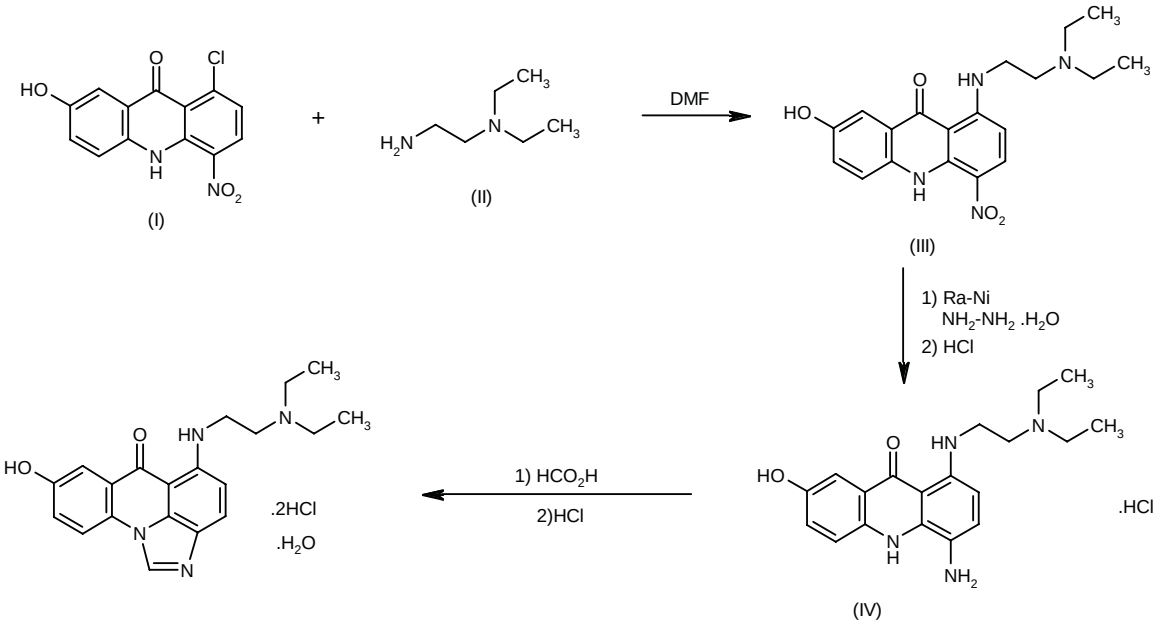
Scheme 1: Synthesis of C-1311



Biological Activity

C-1311 exhibited high antitumor activity *in vivo*. The drug was highly active against transplantable murine tumors such as P388 leukemia (1), and adenocarcinomas of the colon such as MAC15A and MAC29, and the human xenografts HT-29 in nude mice (10). The activity of C-1311 tested against *in vivo* colon carcinomas was compared with 5-FU, the standard agent used in the treatment of colon tumors, and the results are shown in Table I. All the tumors responded to a single dose of 100 mg/kg C-1311 after i.p. administration with statistically significant growth delays of 118-252%. C-1311 produced higher growth delay in MAC15A tumor at a dose of 100 mg/kg than 5-FU at 125 mg/kg.

C-1311 exhibited significant cytotoxic activity against a broad spectrum of tumor cell lines *in vitro*. These included murine and human leukemias, hamster fibrosarcoma and lung carcinoma, and most importantly, solid tumors such as human colon HT-29, KM12, DLD1, SW620 and breast MCF-7 (10-12, NCI Protocols, unpublished data).

The cytotoxic effect of C-1311 was greatly diminished when tested against nondividing primary hepatocytes or confluent C3H/M2 cells, whereas the growth of dividing C3H/M2 was strongly inhibited (11). These data suggest that imidazoacridinones are preferentially active against proliferating cells similarly to pyrazoloacridines (13), as well as antitumor drugs such as doxorubicin, etoposide and amsacrine. Interestingly, C-1311 was equally cytotoxic against fast growing cultures and cells growing in three dimensions as multicellular spheroids. Compared with two-dimensional cultures, cells growing in multicellular spheroids had a slower cell doubling time and a different cell cycle distribution, cytoarchitecture and cell-cell interactions. In contrast to C-1311, classical topoisomerase II inhibitors such as amsacrine, mitoxantrone and doxorubicin were much more cytotoxic against fast growing monolayer cultures compared to cells grown as spheroids (14). This unique activity of C-1311 against cells growing as multicellular spheroids may, at least in part, explain the high activity of this compound against solid tumors *in vivo*.

Imidazoacridinones were readily taken up by living cells and accumulated predominantly in the nucleus (10, 11, 14). The kinetics of drug accumulation differs between cell lines and the maximal intracellular drug level was obtained within 1 h in human leukemia cells, whereas 3 h were required for the maximal drug accumulation in human colon tumor cells (Skladanowski, unpublished). In some cellular systems, imidazoacridinones, including C-1311, were accumulated not only in nuclei but also in cytoplasmic vesicles (10, 14).

C-1311 was shown to be a poor substrate for P-glycoprotein, a plasma membrane efflux pump associated with the multidrug resistance phenotype, which reduces the intracellular concentration of many antitumor compounds. One study utilized a series of P-glycoprotein expressing KB multidrug resistant (MDR) cell lines (15). The low level MDR cell line KB-8.5 (which is 3-fold resistant to doxorubicin) only expressed very slight resistance to C-1311 (1.2-fold). Two more resistant KB lines, KB-A1 and KB-C1 (which are 97- and 160-fold resistant to doxorubicin, respectively) were 2.2-fold and 4-fold resistant to C-1311, respectively. Resistance was reversible by verapamil. In another study the total accumulation of

Table I: Activity of C-1311 in vivo *colon carcinoma models compared with 5-FU as standard agent.*

Tumor	Drug	Dose (mg/kg)	Growth delay (%)	Optimum % T/C (day)
Murine				
MAC15A	C-1311	25	-3.7	57.3 (4)
	C.1311	50	55.5	33.5 (4)
	C-1311	100	251.8	6.5 (4)
	5-FU	125	144	5.6 (4)
MAC29	C-1311	100	117.8	32.1 (10)
	5-FU	125	58.1	40.8 (10)
Human xenograft				
HT29	C-1311	50	68.6	37.8 (18)
	C-1311	100	211.4	21.5 (15)

C-1311 in human myeloid HL-60/Vinc cells, which overexpress a functional P-glycoprotein, was shown to be comparable to that observed in parent HL-60 cells (16). However, the resistant cells were about 10-fold cross-resistant to C-1311 and this was attributed to changes in intracellular drug distribution in HL-60/Vinc cells, with sequestration of the drug in cytoplasmic vesicles. These results are in partial agreement with those obtained for Friend erythroleukemia F4-6/MDR cells, which overexpress P-glycoprotein (11). The total accumulation of imidazoacridinones, including C-1311, in F4-6/MDR cells was about 2-fold lower than in sensitive F4-6/WT cells but the resistance factors obtained for imidazoacridinone compounds were only in the range of 7-13 for cells which are 200-fold resistant to doxorubicin. The cytotoxicity studies as well as the influx and efflux analyses performed in these different cell systems suggest that imidazoacridinones are poor substrates for P-glycoprotein and may be more active than classical drugs against tumor cells with the MDR phenotype. Preliminary experiments using the MRP expressing MDR lung adenocarcinoma cell line, COR-L23/R, suggested that C-1311 may well have an advantage over similar agents in current use (15).

Another promising feature of C-1311 is the lack of oxygen free radicals observed after reduction of the compound by enzymes of the microsomal fraction of rat liver. Therefore, in contrast to adriamycin, imidazoacridinones should not be cardiotoxic (Patterson, unpublished data). It has also been shown that C-1311 exhibited only limited mutagenic potential in V79 Chinese hamster cells and in the *Salmonella* microsome assay (11).

Biochemical Mechanism of Action

C-1311 was shown to bind noncovalently to calf thymus and plasmid DNA as determined by spectrophotometric titration, thermal denaturation and electrophoresis on agarose gels (10, 17). However, no correlation was observed between the ability to bind noncovalently to DNA and cytotoxic and antitumor activity of imidazoacridinones (11, 17). C-1311 was demonstrated to bind irreversibly to DNA of tumor cells, presumably covalently, and previous metabolic activation of the drug was necessary for this binding. In the cell free system, imidazoacridinones bound to DNA after previous enzymatic activation by a horseradish peroxidase/H_2O_2 system (17). In tumor cells treated with C-1311, interstrand DNA cross-linking was observed, and also previous metabolic activation of the drug was a prerequisite for this effect (18).

Since metabolic activation turned out to be the necessary step to covalent binding for C-1311 to DNA, the susceptibility of imidazoacridinones to enzymatic oxidation was studied. These compounds underwent oxidative transformation in the presence of horseradish peroxidase as well as a microsomal fraction of rat liver. A comparison of the level of enzymatic transformations for several imidazoacridinone derivatives with different biological activities suggests that enzymatic oxidation is relevant to the biological and antitumor activity of these compounds. The aminoalkyl side chain as well as the imidazoacridinone core were shown to be the structural elements transformed during the enzymatic oxidation (19). Enzymatic and electrochemical studies with the reference compound, 2-hydroxyacridinone, indicated that the hydroxyl group was involved in the oxidation process and the position *ortho* to the hydroxyl group was the most susceptible to substitution by nucleophilic agents (20). We presume also that nucleophiles such as sulfhydryl groups of proteins or purine and pyrimidine bases of DNA could be easily substituted at position *ortho* to the 8-hydroxy group of C-1311.

Imidazoacridinones, as many other DNA intercalating compounds, inhibited DNA topoisomerases, nuclear enzymes which regulate DNA topology and chromatin function. It was shown that C-1311 and other biologically active imidazoacridinones inhibited the catalytic activity of DNA topoisomerase II. All imidazoacridinones with anticancer activities stimulated the formation of covalent DNA-topoisomerase II complexes (termed “cleavable complexes”) both *in vitro* and in whole cells, whereas inactive compound did not. In addition, DC-3F/9-EOH cells which are resistant to other topoisomerase II

inhibitors, were 30- to 125-fold resistant to active imidazoacridinones. This cross-resistance was associated with a reduction in the formation of DNA-protein complexes and was the highest for compounds which are potent topoisomerase II inhibitors *in vitro* (14). It was also found that imidazoacridinones, including C-1311, inhibited the catalytic activity of DNA topoisomerase I but, in contrast to camptothecin and its analogs, were unable to stabilize cleavable complexes between DNA and topoisomerase I (Skladanowski, unpublished data).

Studies in several cell systems (L1210, HeLaS3, HT-29) indicated that C-1311 induced accumulation of tumor cells in the G_2 phass of the cell cycle (12, 21, 23). This cell cycle effect was induced by a biologically relevant dose of C-1311 corresponding to its EC_{90} value. In L1210 cells the arrest was reversible for up to 3 h of treatment but irreversible at longer incubation times.

L1210 cells treated with C-1311 have been shown to undergo programmed cell death (23). The cell death process was characterized by induction of specific, internucleosomal DNA fragmentation, as revealed by agarose gel electrophoresis and by the morphological changes which are characteristics of apoptosis. C-1311 induced apoptosis in L1210 cells at EC_{90} concentration, at which the drug inhibited cell cycle progression in the G_2 phase. This observation suggests that following G_2 arrest cells spontaneously undergo apoptosis by some as yet unknown mechanism.

Pharmacokinetics

A thorough preclinical pharmacokinetic evaluation of C-1311 was thought to be essential for further development so with this aim in mind a method for quantification of the compound in biological tissues and fluids was developed (24). This high-performance liquid chromatography method using fluorescence detection was shown to be selective, sensitive (limit of detection 1 ng/ml) and reproducible, with recoveries > 90%. This analytical method was used to determine plasma, tissue and tumor pharmacokinetics in mice following intraperitoneal administration of C-1311 in saline at therapeutic doses (25). This study showed that C-1311 is rapidly cleared from the plasma and distributes quickly into tissues and tumors. Plasma pharmacokinetics were linear between doses of 15-100 mg/kg but disproportionate increases were seen in plasma and tissue concentrations at doses above 100 mg/kg. Interestingly, C-1311 distributed unevenly in both mouse and human blood with higher concentrations in the cellular fraction than in the plasma. When it was administered orally to mice, C-1311 concentrations were considerably lower in blood and tissues than those seen with intraperitoneal injection (26). However, significant antitumor effects still occurred, suggesting the potential for oral activity in the clinic.

Preliminary metabolism studies in mice suggest the presence of 8 minor metabolites in the urine with 1 major metabolite (M1-a glucuronide) present in high concentration (27). Further analysis showed high levels of M1 in plasma and liver. A further metabolite M2 was detected at high concentration in liver but not in plasma or urine. This metabolite is undergoing further investigation. The high concentration of glucuronide observed in plasma, liver, urine and feces may be associated with potential enterohepatic recirculation of C-1311, which may be clinically important.

Conclusions

In conclusion, we have shown that the imidazoacridinone drug, C-1311, exhibits biological and biochemical effects which all have impact in antitumor action, although at present it is difficult to identify which of these effects are crucial and responsible for the high antitumor potency of the drug. The attractive pharmacological properties of C-1311 include 1) preferential cytotoxic and antitumor activity against solid tumors, including xenograft of human colon carcinoma HT-29 in nude mice; 2) inability of induction of oxygen free radicals suggesting lack of cardiotoxic properties; 3) rapid accumulation in tumor cells, especially in the nucleus; 4) induction of apoptosis in tumor cells without irreversible inhibition of DNA synthesis; 5) activity against MDR expressing cell lines; 6) active after oral administration; and 7) low mutagenic potential.

Source

Technical University of Gdansk (PL).

References

1. Cholody, W.M., Martelli, S., Konopa, J. *Chromophore-modified antineoplastic imidazoacridinones. Synthesis and activity against murine leukemias.* J Med Chem 1992, 35: 378-82.

2. Capps, D.B. (Warner-Lambert Co.). *Pyrazolo[3,4,5-k]acridine cpds., pharmaceutical compsns. containing the same and processes for their production.* EP 138302.

3. Cheng, C.C., Zee-Cheng, R.K.Y. *The design, synthesis and development of a new class of potent antineoplastic anthraquinones.* In: Progress in Medicinal Chemistry, G.P. Ellis and G.B. West (Eds.), Elsevier Science Publishers, B.V., New York, 1989, 83-118.

4. Konopa, J., Skladanowski, A. *Mitoxantrone induces covalent interstrand DNA crosslinking in tumor cells.* In: Recent Advances in Chemotherapy, J. Ishigami (Ed.), University of Tokyo Press, Tokyo, 1985, 633-4.

5. Konopa, J. *Interstrand DNA crosslinking in tumor cells by 1-nitroacridines, anthracyclines and aminoanthraquinones.* Pharmacol Ther 1990, 7(Suppl.): 83-94.

6. Cholody, W.M., Martelli, S., Lukowicz, J., Konopa, J. *5-[(Aminoalkyl)amino]-imidazo[4,5,1-de]acridin-6-ones as a novel class of antineoplastic agents. Synthesis and biological activity.* J Med Chem 1990, 33: 49-52.

7. Cholody, W.M., Martelli, S., Konopa, J. *8-Substituted 5-[(aminoalkyl)amino]-6H-v-triazolo[4,5,1-de]acridin-6-ones as potential antineoplastic agents. Synthesis and biological activity.* J Med Chem 1990, 33: 2852-6.

8. Konopa, J., Wysocka-Skrzela, B., Horowska, B. *4-Substituted 1-alkylenediaminoacridones as potential antitumor agents.* 7th NCI-EORTC Symp New Drugs Cancer Ther (March 17-20, Amsterdam) 1992, Abst 119.

9. Cholody, W.M., Horowska, B., Paradziej-Lukowicz, J., Martelli, S., Konopa, J. *Structure-activity relationship for antineoplastic imidazoacridinones: Synthesis and antileukemic activity in vivo.* J Med Chem 1996, 39: 1028-32.

10. Burger, A.M., Double, J.A., Konopa, J., Bibby, M.C. *Preclinical evaluation of novel imidazoacridinone derivatives with potent activity against experimental colorectal cancer.* Brit J Cancer 1996, 74: 1369-74.

11. Berger, B., Marquardt, H., Westendorf, J. *Pharmacological and toxicological aspects of new imidazoacridinone antitumor agents.* Cancer Res 1996, 56. 2094-104.

12. Augustin, E., Wheatley, D.N., Lamb, J., Konopa, J. *Imidazoacridinones arrest cell-cycle progression in the G2 phase of L1210 cells.* Cancer Chemother Pharmacol 1996, 38: 39-44.

13. Jackson, R.C., Sebolt, J.S., Shillis, J.L., Leopold, W.R. *The pyrazoloacridines: Approaches to the development of a carcinoma-selective cytotoxic agent.* Cancer Invest 1990, 8: 39-47.

14. Skladanowski, A., Plisov, S.Y., Konopa, J., Larsen, K.L. *Inhibition of DNA topoisomerase II by imidazoacridinones, new antineoplastic agents with strong activity against solid tumors.* Mol Pharmacol 1996, 49: 772-80.

15. Warr, J.R., Quinn, D.M., Double, J.A., Bibby, M.C. *Low level multidrug resistance expression to C-1311, a potential clinical candidate.* Brit J Cancer 1997, 75: 33.

16. Skladanowski, A., Come, M.G., Laurent, G., Konopa, J., Larsen, A.K. *PSC-833 and tubulin interacting agents reverse the altered drug distribution in multidrug resistant HL-60 cells as revealed by the fluorescent antitumor drug imidacrine.* Proc Amer Assoc Cancer Res 1997, 38: Abst 3990.

17. Dziegielewski, J., Skladanowski, A., Konopa, J. *Noncovalent binding of potent antitumor imidazoacridinones to DNA.* Ann Oncol 1996, 7(Suppl. 1): 56.

18. Dziegielewski, J., Konopa, J. *Interstrand crosslinking of DNA induced in tumor cells by a new group of antitumor imidazoacridinones.* Proc Amer Assoc Cancer Res 1996, 37: Abst 2800.

19. Mazerska, Z., Gorlewska, K., Kraciuk, A., Konopa, J. *The relevance of enzymatic oxidation by horseradish peroxidase to antitumor potency of imidazoacridinone derivatives.* Chem Biol Interact 1998, in press.

20. Mazerska, Z., Zamponi, S., Marassi, R., Martelli, S., Konopa, J. *Electrochemical oxidation of antitumor imidazoacridinone derivatives and the reference 2-hydroxyacridinone.* J Electroanal Chem 1997, 427: 71-8.

21. Wheatley, D.N., Lamb, J. *Cell killing by the novel imidazoacridinone antineoplastic agent, C-1311, is inhibited at high concentration coincident with dose-differentiated cell cycle perturbation.* Brit J Cancer 1996, 74: 1359-61.

22. Augustin, E., Konopa, J. *Imidazoacridinones arrest cell-cycle progression in the G_2 phase of the HT29 cells.* J Comp Assist Microsc 1997, 9: 21.

23. Augustin, E., Konopa, J. *Imidazoacridinones induce apoptosis in murine leukemia L1210 cells.* Fol Cytochem Cytobiol 1996, 34(Suppl. 2): 56.

24. Calabrese, C.R., Loadman, P.M. *Analysis of the imidazoacridinone C1311 by high-performance liquid chromatography.* J Chromatogr B 1997, 690: 275-81.

25. Calabrese, C.R., Bibby, M.C., Double, J.A., Loadman, P.M. *Pharmacokinetics and tissue distribution of the imidazoacridinone C-1311 in tumor bearing mice.* Cancer Chemother Pharmacol 1998, in press.

26. Calabrese, C.R., Loadman, P.M., Bibby, M.C., Double, J.A. *Anti-tumor activity and pharmacokinetics following oral administration of C-1311.* Proc Amer Assoc Cancer Res 1997, 38: Abst 2057.

27. Calabrese, C.R., Loadman, P.M., Bibby, M.C., Double, J.A. *Assessment of murine metabolism of the imidazoacridinone C-1311.* Brit J Cancer 1997, 75(Suppl. 1): 22.