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Review paper

Acronycine derivatives as promising antitumor agents

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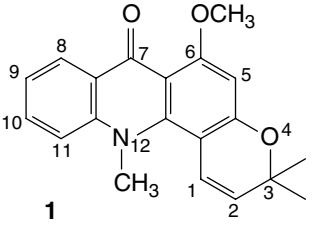
Originally isolated from an Australian plant, acronycine is an antitumor alkaloid with poor water solubility and low potency. The modest antitumor activity of this compound was markedly improved by the total synthesis of original analogs resulting in the selection of S23906-1, a diester derivative of 1,2-dihydrobenzo[*b*]acronycine. S23906-1 is characterized *in vitro* by a high potency in clonogenic assays and uncommon cell cycle pertubations. *In vivo*, this compound demonstrated a selectivity for human solid tumors as compared to murine transplantable tumors. The unique pharmacological profile of S23906-1 was particularly defined by a broad antitumor efficacy when administered i.v. or orally on aggressive orthotopic models of human lung, ovarian and colon models with comparable or better activity than clinically used anticancer drugs. The molecular mechanism of action of S23906-1 could involve DNA alkylation, modulation of cyclin E protein levels and inhibition of DNA synthesis leading to apoptosis. Ongoing preclinical toxicological studies will help to define the potential of this novel agent which is already considered as a valuable candidate for clinical studies. [© 2002 Lippincott Williams & Wilkins.]

Key words: Acronycine, cell cycle, cytotoxic drug, human tumors, orthotopic models, S23906-1.

Acronycine

Despite the considerable progress made in target-based drug design and combinatorial chemistry, a majority of anticancer drugs currently used in the clinic are derived from natural compounds primarily selected in screening systems. In some well-documented examples, such as vinca alkaloid or camptothecin derivatives, the optimization process has been excessively long but resulted in major improvements in cancer therapy. Acronycine (**1**) is a natural pyranoacridone alkaloid, which was first isolated in 1948 from the stem bark of a small Australian Rutaceous tree, *Acronychia baueri* Schott (=*Sarcomelicope simplicifolia* (Endl.) Hartley).[1,2] Its angular 3,12-dihydro-6-methoxy-3,3,12-trimethyl-7*H*-pyrano-[2,3*c*]acridine-7-one structure was established by Macdonald and Robertson in 1966.[3] As is often the case with compounds isolated from natural sources, an initial antitumor evaluation was performed with a crude extract from the shrub bark, but this failed to show any activity in a primary screen, the murine P1534 leukemia. Subsequently, Svoboda and colleagues demonstrated that, after a differential extraction technique followed by purification and crystallization, acronycine exhibited antitumor properties in a panel of murine tumor models including S-180 and AKR sarcomas, X-5563 myeloma, S-115 carcinoma, and S-91 melanoma. These studies also revealed the marginal activity of the drug when administered by the i.v. route as compared to i.p. or oral routes, partly due to its poor solubility in aqueous solvents.[4] Co-solvent systems consisting of PEG 300, ethanol and Polysorbate 80 were used to perform parenteral administration of acronycine.[5] However, in the ensuing pharmacological assays, the antitumor efficacy of acronycine was not markedly



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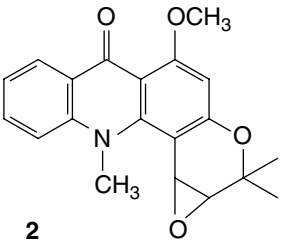
improved.[5,6] Based on the significant but moderate activity demonstrated against the murine tumor models mentioned above, acronycine was formulated in oral capsules and evaluated in a limited phase I–II clinical trial in patients with multiple myeloma.[7] In this study, one partial response was observed in a patient treated for a total of 93 weeks up to a maximum daily dose of 400 mg/m^2, but the treatment was associated with significant gastrointestinal and neurological toxicities.

Numerous studies have been carried out to better understand the molecular mechanism of action of acronycine, but with a limited success. Clearly, the large coplanar chromophore of this drug would suggest a likely interaction with DNA through intercalation. Consistent with this hypothesis, Dorr and Liddil have demonstrated that acronycine could inhibit thermal denaturation of calf DNA, suggesting a non covalent binding between the drug and the double helix.[5] Others have documented an indirect inhibition of RNA and DNA synthesis, resulting from an alteration of the cellular uptake of nucleosides.[8] Several conflicting reports on the cell cycle effects of acronycine have also successively described arrest of treated cells in G_1+G_2+M or S+G_2+M phases of the cell cycle.[9,10] Despite the lack of consensus concerning the mechanism of action of acronycine, its cytotoxic activity, even if observed at relatively high concentrations, has been demonstrated using human cancer cell lines or tumor explants cultured *in vitro* as well as human tumor xenografts *in vivo*.[6]

Design of improved acronycine derivatives

All the data reported above suggest that acronycine is an interesting compound whose potency and antitumor activity could probably be markedly improved through modification of its chemical structure. Consequently, an important effort was devoted to the search for bioactive synthetic or natural structural analogs. Disappointingly, none of the compounds prepared during the first 25 years following the description of the biological properties of acronycine presented a better profile than the parent compound.[1,11] Nevertheless, these results permitted us to delineate the structural features indispensable to the pharmacological activity. From this point of view, the dimethylchromene system corresponding to the C and D rings was shown to play an essential role. In this pharmacophore, the 1,2-double bond appeared as an indispensable structural requirement for



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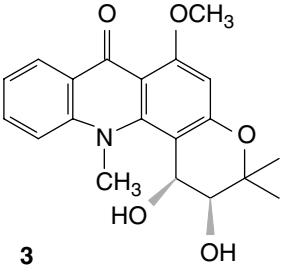
cytotoxic and antitumor activities.[1] The presence of a methoxy electron-donating group at C-6 was also clearly important.[1]

Meanwhile, acronycine epoxide (**2**) was isolated in minute amounts from *Sarcomelicope argyrophylla* Guill., in the course of a systematic study of the alkaloids of plants belonging to the genus *Sarcomelicope*.[12,13] The isolation, characterization and synthesis of this compound proved extremely difficult, in connection with its high chemical unstability.[13–16] For instance, **2** readily reacted with water, acting as a nucleophile, to give the corresponding *cis* and *trans* diols **3** and **4**. The high reactivity of the oxirane group led to the speculation that acronycine epoxide should be the active metabolite of acronycine *in vivo* and that it should be responsible for the alkylation of nucleophilic targets within the tumor cell.[13] This was a key observation and the hypothesis of bioactivation was in full agreement with the importance of the 1,2-double bond in the biological activity. In this context, 1,2-dihydroxy-1,2-dihydroacronycine diesters appeared as possible new anticancer candidates. Such compounds were expected to present a better chemical stability than acronycine epoxide, but a similar reactivity at the benzylic 1-position toward nucleophilic agents.[17–19]



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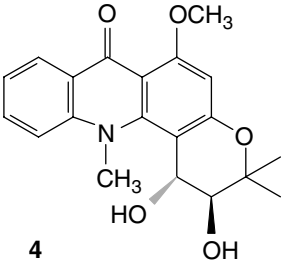

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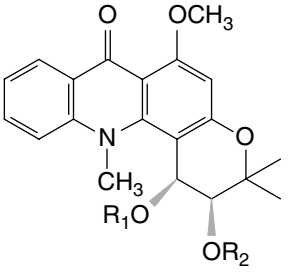
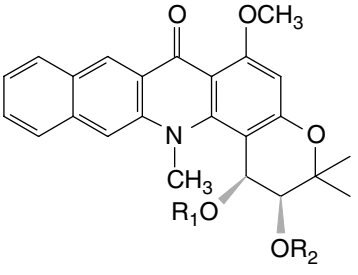


5 $R_1 = R_2 = COCH_3$

Indeed, 1,2-dihydroxy-1,2-dihydroacronycine diesters, exemplified by *cis*-diacetate **5**, exhibited interesting antitumor properties and an increased potency when compared with the parent alkaloid against L1210 leukemia cell line *in vitro*.[17] Demonstration of an interaction of acronycine with DNA[5] was conducted to design a series of 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters, fused on the natural alkaloid basic skeleton.[20] Indeed, interaction with DNA is known to occur mainly for compounds with sufficiently large co-planar aromatic chromophores in several related series of antitumor drugs such as acridines, pyridocarbazoles and anthracene-diones. The new benzo[*b*]acronycine derivatives were even more potent and more active *in vitro* and *in vivo* against the murine C38 colon adenocarcinoma than both acronycine and 1,2-dihydroxy-1,2-dihydroacronycine diesters, and the most promising derivative S23906-1, the diacetate **6**, was selected for further preclinical development.[21]

Preclinical antitumor activity of S23906-1

A unique pharmacological profile for S23906-1 that has been characterized *in vitro* as well as *in vivo* makes the compound quite different from existing anticancer drugs.[22,23] S23906-1, previously shown to be cytotoxic for cancer cells, was surprisingly more potent in a clonogenic assay than in the standard proliferation assay even after a short-duration exposure of 1 h. Flow cytometric analysis of cell cycle effects were also quite uncommon for a cytotoxic drug and depended on the concentration used. Treatment with 1 μM S23906-1 resulted in a partially reversible accumulation of HT-29 colon cancer cells in the G_2+M phases of cell cycle, whereas higher concentrations induced an irreversible arrest of the cells in the S phase. These modifications in cell cycle parameters were preceded by a rapid inhibition of DNA synthesis and were followed by induction of apoptosis detected by Annexin V labeling.[22] It should be noted that, under the same experimental conditions, no significant perturbation of the cell cycle could be detected with 50 μM acronycine.



6 $R_1 = R_2 = COCH_3$

7 $R_1 = R_2 = H$

The level of predictivity of *in vivo* models used to evaluate anticancer drugs remains at the present time an open question and the relative importance of orthotopic models as compared to classical murine cancer model systems is of particular interest.[24–26] For this reason, the evaluation of the antitumor activity of S23906-1 was performed in the two types of models. A first set of experiments was designed in the murine former National Cancer Institute panel including P388 leukemia, B16 melanoma, Lewis lung carcinoma and C38 colon adenocarcinoma, in order to assess the antitumor activity of S23906-1 and the effect of different routes and schedules of administration. Globally, the drug proved to be poorly active in this panel, except against the C38 colon adenocarcinoma in which S23906-1 administered twice i.v. from 1.56 to 6.25 mg/kg markedly inhibited tumor growth and induced tumor regression at the highest dose. In the same experiment, acronycine was 16-fold less potent and only moderately active at the maximal tolerated dose, 100 mg/kg i.v. These results confirmed the reported low potency of acronycine and the markedly improved antitumor properties for S23906-1 in C38 colon carcinoma model in terms of activity, potency and therapeutic index. This impressive antitumor efficacy demonstrated by S23906-1 led to its selection for further preclinical evaluation. Moreover, its pharmacological activity was associated with moderate body weight losses which were dose dependent (12% of the weight of mice treated at 6.25 mg/kg) and reversed within 10 days after drug administration. This suggests an acceptable toxicological profile for S23906-1, at least in the mouse.

In order to define the efficacy of S23906-1 toward human cancers, a second set of experiments involving aggressive models of human ovarian,[27] lung[28] and colon carcinomas[29] was envisaged. Surprisingly, S23906-1 demonstrated significant antitumor activity when administered i.v. with a similar level of efficacy

than that of clinically used drugs.[23] Against the ovarian (IGROV1 and NIH:OVCAR-3) and non-small cell lung tumors (NCI-H460 and A549), S23906-1 administered twice i.v. at 1.56–6.25 mg/kg increased the survival of tumor-bearing mice in a dose-dependent manner, being even curative in the NIH:OVCAR-3 mode, and was as efficient as paclitaxel. Whereas against IGROV1 and NCI-H460 tumors, while acronycine proved to be only marginally active at 100 mg/kg i.v., S23906-1 at 6.25 mg/kg induced T/C values of 193 and 162%, respectively. Remarkably, this high level of antitumor activity was maintained when S23906-1, formulated in Solutol HS 15, was given orally at 12.5 and 25 mg/kg following the same treatment schedule, suggesting good bioavailability. Other different and complementary parameters, such as primary tumor growth, loco-regional invasion and distant organ metastasis, were used to evaluate the efficacy of S23906-1 against intracecally grafted HT29 and HCT116 colon cancers. Histological studies allowed us to demonstrate that the drug inhibited the growth of primary tumors located in the cecum of animals as efficiently as irinotecan, but more importantly eradicated the formation of lymph node, hepatic and pulmonary metastasis in the aggressive HCT116 model. Interestingly, compound **7**, one of the close analogs of S23906-1 which lacks the two acetate groups, proved to be totally inactive in the same orthotopic models.[30]

The rationale on which these new acronycine derivatives have been synthesized, based on the epoxide hypothesis, as well as the cytotoxic effects seen after a short-duration exposure to the drug are both in favor of an alkylating mechanism for S23906-1. Retardation gel and DNase footprinting experiments identified recently the guanine N^2 in the DNA minor groove as the potential reactive site.[31] It remains to be seen whether this unusual mode of DNA alkylation is totally responsible for the cell cycle and cytotoxic effects of S23906-1. Additional biochemical data, i.e. the rapid and net increase in cyclin E protein level as well as the complete inhibition of BrdU incorporation induced by S23906-1 have recently been documented.[22] However, the complete sequence of events leading to cellular apoptosis remains to be identified. The establishment of a cell line made 15-fold resistant to S23906-1 by stepwise exposure to the drug and that still accumulates in S phase but without any increase in cyclin E protein level after treatment may help to gain some insight into the mechanism of action of this new cytotoxic compound.[22]

Before a new promising anticancer agent enters clinical development, several hurdles have to be overcome. In addition to the demonstration of strong evidence of antitumor activity in relevant preclinical models in mice, a relatively safe toxicological profile in superior species and satisfying pharmacokinetic parameters have to be met. Finally, the design of a formulation compatible with clinical administration in man is also desirable. All these issues are currently under active investigation and will define the potential of S23906-1 as a clinical candidate.

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