HIV-1 Neutralization and Tumor Cell Proliferation Inhibition in Vitro by Simplified Analogues of Pyrido[4,3,2-*mn*]thiazolo[5,4-*b*]acridine Marine Alkaloids

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The antiviral/antitumor marine alkaloid dercitin was used as a lead compound to design analogues with anti-HIV and tumor inhibitory activities. Deletion of structural features contributing to cytotoxicity led to analogues with lowered T-lymphocyte toxicity profiles. One compound, **5**, induced complete protection against HIV-1 infectivity in vitro at 12.5 μg/mL (38 μM) without T-cell toxicity up to 400 μg/mL. Compound **4** and **5** also inhibited the binding of HIV-1 to H-9 lymphocytes. These compounds may exert antiviral activity by a unique dual extracellular and intracellular mode of action—both preventing viral attachment to lymphocytes as well as intercalating with viral nucleic acid. Analogues with higher cytotoxicity such as **2** which retain the thiazole ring of the natural product proved effective in completely inhibiting the cell proliferation of breast, colon, and lung tumor cell lines at 1.5 μM concentration compared to a 70 μM dose level of 5-fluorouracil. A means of molecular separation of antiviral activity from cytotoxicity was thus achieved, and putative pharmacophores for antiviral and antitumor actions of the prototype molecule dercitin have been deduced. The 2-thio-9-acridinone derivatives **4** and **5** represent a new structural type exhibiting activity against HIV in vitro, serving as chemical leads in the design of anti-AIDS agents, while thiazolo[5,4-*b*]acridines such as **2** provide leads in the drug design of new antitumor agents.

Introduction/Rationales

Marine fauna species from coral reef habitats represent a largely untapped resource for the identification of new non-nucleoside antiviral agents. Sponges, corals, and tunicates are sessile animals, which, in the absence of physical defenses, have evolved chemical defense systems to ward off the proliferation of rival species in the reef substrata around them. These same properties of inhibiting cell division of competing organisms have been found to impart to the marine alkaloids antitumor and antiviral activities as well.

The prototype molecule possessing both antitumor and antiviral activities is dercitin, **1a** (Chart I). It possesses the structurally distinct pyrido[4,3,2-*mn*]thiazolo[5,4-*b*]-acridine nucleus unknown in any terrestrial natural product. This is a near-planar pentacyclic structural moiety with a flexible (dimethylamino)ethyl side chain at the C-4 position. Dercitin is obtained from a sponge of the genus *Dercitus* (Family Pachastrellidae) from a reef in the Bahamas.[1] It was found to have antiviral activity against both a DNA virus, herpes simplex virus type 1 (HSV-1) (50% inhibition at 5 μg/mL), and an RNA virus, murine coronavirus (100% inhibition at 1 μg/mL) and against a host of tumor cell lines (IC_{50} for P388 leukemia cells = 0.05 μg/mL).[1,2] It was also found effective against tumor cell lines that expressed multidrug resistance. Dercitin therefore represents a natural molecule which provides a good structural lead for non-nucleoside antiviral drug design, but is in all probability a poor clinical candidate because it inhibits viral replication at concentrations too close to its cytotoxic dose for mammalian cells. Other related marine alkaloids isolated from *Dercitus* and a related sponge *Stelleta* include dercitamide (**1c**) and nordercitin (**1b**) which differ in the nature of the C-4 side-chain substituent.[3] A related alkaloid, kuanoniamine A, which has an isomeric pyrido[4,3,2-*mn*]thiazolo[4,5-*b*]-acridine structure isolated from an unidentified Pacific tunicate, has also been reported to inhibit the proliferation of KB nasopharyngeal cancer cells.[4]

The purpose of the present study was an attempt to identify the pharmacophores within the structure of dercitin that impart antiviral activity and confer mammalian cytotoxicity, respectively. It was postulated that it may be possible to design structurally simplified congeners of dercitin which have a wider therapeutic index (ratio of EC_{50}:IC_{50}) and possess activity against HIV-1 without concomitant cytotoxicity.

In the case of dercitin, there is strong evidence that it exerts its antiviral and antitumor actions by intercalating with nucleic acid.[2] It has a planar fused ring structure similar to the antimalarial/antineoplastic drug quinacrine and the antitumor drug adriamycin, both well-known intercalating agents. It also shows similarities in structure to the plant alkaloid ellipticine, currently being evaluated as an antitumor agent.[5] The present experiments are part of a larger study of structural congeners of dercitin and kuanoniamine as potential antiviral and antitumor agents and consisting of evaluation of the structural features of dercitin which contribute to the anti-HIV activity, T-cell toxicity, and tumor-cell toxicity.

It was surmised that simplified fused ring analogues of dercitin, in which the planar tricyclic system ABC or tetracyclic ABCD responsible for DNA intercalation was retained but in which other structural features which contribute to cellular toxicity were deleted, may yield compounds with higher antiviral therapeutic indexes.

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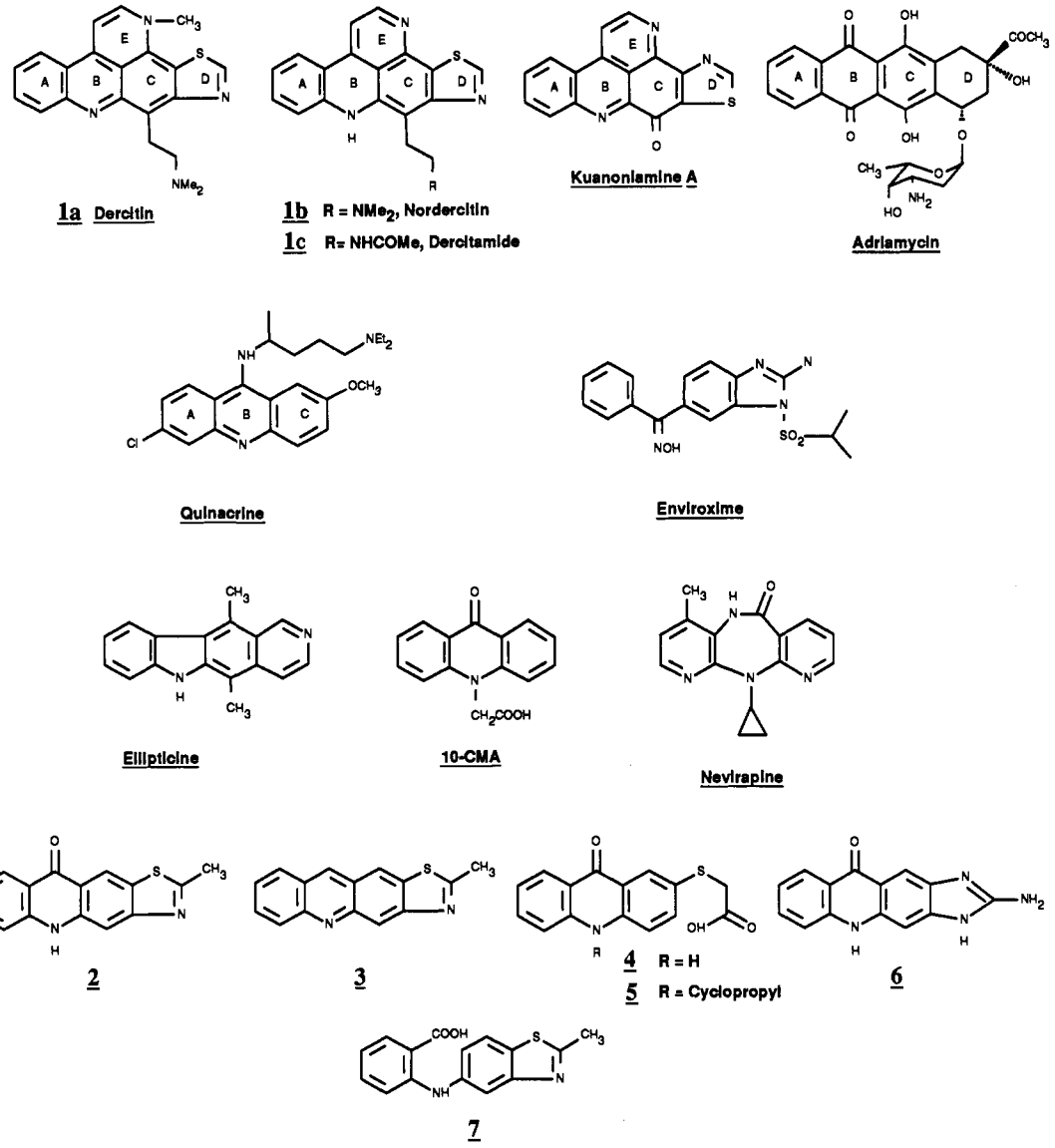
Chart I



Accordingly, the analogues 2–7 which incorporate dercitin substructures were synthesized and evaluated for anti-HIV activity and cytotoxicity to MT-4 lymphocytes and human peripheral blood mononuclear cells (PBMC).

A clinically useful anti-HIV agent should inhibit virus replication but not be cytotoxic to lymphocytes. Intercalation with double-stranded nucleic acid requires the insertion of a flat molecule between base-pairs in a double helix. This causes the subsequent inhibition of DNA topoisomerases in mammalian systems or of RNA polymerases in both prokaryotes and eurkayotes.[6] Frame-reading errors may then ensue in transcribed mRNA, resulting in inhibition of protein synthesis. Retroviral replication of HIV-1 could be inhibited by an agent which intercalates with the reverse transcriptase (RT) induced DNA transcript of viral genomic RNA.

The dercitin molecule has four basic nitrogen atoms. These nucleophilic centers in the structure could produce significant secondary cytotoxic effects on mammalian cells causing the molecule to bind to acidic amino acid residues in cellular structural proteins and enzymes and also have nonintercalative interactions with phosphate residues of nucleic acids. This may result in cytotoxic effects to the mammalian cells unrelated to an intercalative mechanism of action.

The analogues 2 and 3 represent compounds in which the nonlinearly fused pyridine ring and the basic (dimethylamino)ethyl side chain of dercitin have been eliminated to simulate the ABCD ring system of adriamycin or ellipticine. Compound 4 was previously prepared by Taraporewala and Kauffman[7] as a potential anti-inflammatory agent; it appeared to be a good candidate for antiviral evaluation as well, having a sulfur atom attached to an acridine nucleus similar to dercitin and also possessing an acetic acid side-chain moiety resembling that of 10-(carboxymethyl)-9-acridinone (10-CMA), a known antiviral agent which is one of the most potent low molecular weight inducers of interferons from macrophages and leukocytes.[8,9] Interferons are the body's endogenous

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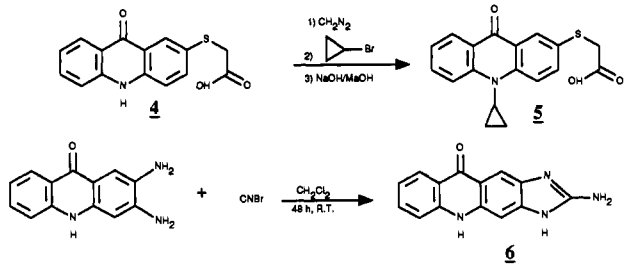
Scheme I



antiviral protein substances which impart resistance to viral infection in these cells. Compound **5** incorporates an *N*-cyclopropyl group into the tricyclic system of **4**, similar in structure to the recently reported anti-HIV RT-inhibitor, nevirapine.[10,11] Compound **6** has an imidazo[4,5-*b*]acridine structure wherein an imidazole ring replaces the thiazole ring of dercitin. It also represents a bridged version of the antiviral agent, enviroxime, which has a broad spectrum of activity against picornaviruses.[12] Compound **7** is an analogue which cannot intercalate as it cannot assume a ring coplanar conformation.

The analogues **2** and **3** most resemble the known antitumor agents adriamycin (doxorubicin) and ellipticine in having a linearly fused tetracyclic ring structure which is coplanar and capable of DNA intercalation (Chart I).

The test compounds derived by a strategy of structural elision therefore represent a good model system for deducing the structural features of the prototype dercitin molecule responsible for antiviral and cytotoxic activities and for evaluating the putative pharmacophores for these respective actions. These structure–activity relationships should provide chemical leads towards the design of novel antitumor and antiviral agents.

Chemistry

The syntheses of **2**, **3**, and **7** have been recently reported.[13] Compound **4** was prepared as previously described.[7] Compound **5** was synthesized (Scheme I) from **4** by its esterification with diazomethane in ether followed by treatment with sodium hydride (1.2 equiv) in *N*,*N*-dimethylformamide and bromocyclopropane (1.2 equiv) at 60 °C, and an in situ saponification of the resultant methyl ester; overall yield was 64%. Compound **6** was prepared by treatment of 2,3-diamino-9-acridinone[14] with excess cyanogen bromide (room temperature, dichloromethane solvent) in a procedure analogous to that reported for the synthesis of enviroxime[12] (Scheme I).

Table I. Cytotoxicity and HIV-1 Neutralization in MT-4 Lymphocytes

compound	concentration μg/mL	concentration μmol/mL	cytotoxicity: % viability	HIV-1 neutralization: 7-day % viability
control cells	–	–	96.9	78.9
control cells + HIV-1	–	–	–	18.6
2[a]	2	0.0076	91.9	–
	10	0.0375	87.2	19.4
	100	0.3750	74.3	–
3[a]	2	0.0080	85.0	–
	10	0.0400	78.3	14.3
	100	0.4000	57.1	–
4[b]	2	0.0070	96.7	–
	10	0.0350	93.3	–
	100	0.3500	92.6	70.9
5[b]	2	0.0060	96.6	–
	10	0.0300	96.0	–
	100	0.3000	100.0	83.7
6	2	0.0060	90.0	–
	10	0.0290	88.8	–
	100	0.2900	81.5	12.5
7[b]	2	0.0070	87.5	–
	10	0.0350	92.6	–
	100	0.3500	93.3	18.2
quinacrine dihydrochloride	2	0.0040	93.6	–
	10	0.0200	88.2	–
	100	0.2000	82.1	0.0

[a] Tested as hydrochloride salt. [b] Tested as sodium salt.

Biological Results and Discussion

Test compounds were evaluated first for their cytotoxic effects in cultured MT-4 lymphocytes (Table I). Results indicated that compounds **2**, **3**, and quinacrine (representing a cytotoxic acridine derivative control) displayed cytotoxicity at 10 μg/mL and **6** at 100 μg/mL. The remaining test compounds were noncytotoxic at 100 μg/mL, the maximal evaluated dose.

HIV-1 neutralization was then evaluated in infected MT-4 cells, and the inhibition of the cytopathic effects produced by the test compounds was measured by determination of the percentage of viable cells post-infection with HIV-1 (Molt IIIB strain) at 4 and 7 days subsequent to dosing with a single dose of test compound. Compounds **2**, **3**, and quinacrine (the most cytotoxic compounds) were evaluated at 10 μg/mL and the remaining compounds at 100 μg/mL. Results of this assay are shown in Table I. Compounds **2**, **3**, **6**, and **7** failed to protect MT-4 cells against HIV-1 infectivity. However, **4** and **5**, which are tricyclic analogues with a mercaptoacetic acid side chain, provided good protection against virus at 0.35 μmol/mL and 0.29 μmol/mL, respectively, which represent noncytotoxic concentrations for the MT-4 lymphocytes.

Compounds **4** and **5** were therefore selected for further study of anti-HIV activity. Table II shows the dose-related HIV-1 neutralization in MT-4 lymphocytes following single doses of compounds. Compound **4** produced consistent inhibition of HIV-1 infectivity at >100 μg/mL with no manifestation of cytotoxic effects at 400 μg/mL, the highest evaluated dose. Compound **5** produced complete protection of HIV-1 infectivity in vitro at 12.5 μg/mL (0.038 μmol/mL) with no cytotoxicity seen up to 400

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Table II. Dose-Related HIV-1 Neutralization in MT-4 Lymphocytes by Analogues 4, 5, and AZT

compound/test system	drug concn μg/mL	drug concn μmol/mL	HIV-1 neutralization: % survival of MT-4 cells	HIV-1 neutralization: MTT assay[a] HIV-Molt IIIB strain	HIV-1 neutralization: MTT assay[a] HIV-MN strain
control	-	-	86.0	-	-
control + virus	-	-	13.6	0[a]	0
control + virus + 4	100	0.35	72.7	21.0	13.1
	200	0.70	81.4	100.0	40.5
	400	1.40	84.2	74.3	25.0
control	-	-	84.8	-	-
control + virus	-	-	42.8	0	0
control + virus + 5	12.5	0.038	77.3	-	-
	25	0.075	85.5	-	-
	50	0.150	89.2	-	-
	100	0.30	83.7	3.9	11.3
	200	0.60	81.3	23.5	10.0
	400	1.20	86.0	23.3	11.6
control	-	-	82.6	-	-
control + virus	-	-	7.2	-	-
control + virus + AZT	0.008	0.00003	74.3	-	-
	0.016	0.00006	79.4	-	-
	0.032	0.0012	78.3	-	-

[a] Inactivation determined as outlined in Experimental Section.

Table III. Inhibition of HIV-1 Binding to H-9 Cells

compound/test system	concn (μg/mL)	% bound	% inhibn
control	0	4.9	-
control + virus	0	33.3	-
control + virus + 4	100	22.4	32.7
control + virus + 5	100	28.7	13.8

μg/mL 4 days post-infection. Solubility limitations precluded cytotoxicity testing at concentrations exceeding 400 μg/mL. The zidovudine (AZT) standard in the assay was considerably more potent than 4 or 5.

HIV-1 inhibition was also assayed in a tetrazolium dye uptake microtiter assay (MTT assay) using a colorimetric technique, against two strains of HIV-1, Molt IIIB and HIV_{MN}, respectively. While there were quantitative differences in inhibitions produced by 4 and 5 on the two viral strains, both types were inhibited following infection of MT-4 lymphocytes (Table II).

Binding of virus to H-9 lymphocytes was studied using a fluorescence antibody technique[15] using a concentration of 100 μg/mL of each compound, 4 and 5. Partial inhibition of viral binding to the lymphocytes was observed (Table III). Thus, their antiviral effects may in part be due to an extracellular mechanism of action. Compounds 4 and 5 show structural features in common with the known inducer of interferons, 10-CMA[8] (Chart I), and it is possible that they also have similar effects. A γ-interferon assay on compounds 4 and 5 was carried out by radioimmunoassay (RIA) using dose ranges of 100–400 μg/mL on MT-4 lymphocytes. However, neither produced any significant induction of γ-interferon (data not shown). However, this does not preclude the possibility that they may induce other forms of interferon; β-interferon is the predominant type induced in lymphocytes, while γ-interferon is the major type in macrophages.[16] Effects of test compounds on induction of α- and β-interferons remains to be evaluated.

Table IV. HIV-1 Inhibition and Cytotoxicity in Human PBMC

compound	HIV-1 inhibition:[a] EC_{50} μg/mL	HIV-1 inhibition:[a] EC_{50} μM	cytotoxicity: IC_{50} (μM)
2	22.6	84.6	21.1
3	NE[b]	NE[b]	16.8
4	>28.6	>100 (31)	>100 (15)
5	>32.5	>100 (29)	>100 (9)
6	>34.5	>100 (33)	83.5
7	>7.44	26.04	89.6
AZT	0.273	0.004	>100
quinacrine	NE[b]	NE[b]	2.15
9-aminoacridine	NE[b]	NE[b]	2.14
3,6-diaminoacridine	NE[b]	NE[b]	>1.0
10-CMA, K-salt	>27.7	>100 (29)	>100 (13)

[a] Numbers in parentheses indicate % inhibition observed at 100 μM. [b] Not evaluated for HIV-1 inhibition due to high cytotoxicity.

Compounds were also evaluated against HIV-1 in peripheral blood monocyte cells (PBMC) and for their cytotoxic effects in these cells. Results (Table IV) indicate that the compounds were somewhat less effective in viral inhibition in these cells relative to $CD4^+$MT-4 lymphocytes and also considerably less potent than the AZT standard. However, the cytotoxicities of all the analogues were lower than those of several commercial acridine derivatives such as quinacrine and proflavine used in the assay as well. In the PBMC assay, the nontricyclic analogue 7, also showed HIV-1 inhibition at a noncytotoxic concentration.

The partial inhibition of viral binding to lymphocytes may not fully account for the HIV-1 infectivity neutralization produced by 4 and 5. Since dercitin[2] and quinacrine are known DNA-intercalating agents, it seemed possible that the analogues 4 and 5 exhibit such activity as well. Preliminary DNA-intercalation studies were performed on 2, 3, 4, and 5 using a spectrofluorometric technique.[17] When DNA-intercalative agents interact with double-stranded DNA, a concentration-dependent quench in the

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fluorescence intensity is observed together with a bathochromic shift in the fluorescence emission wavelength. They also displace bound ethidium bromide from DNA in a concentration-dependent manner. All the test compounds 2, 3, 4, 5, and 6, showed these effects on calf thymus DNA and *Micrococcus lysodeikticus* DNA, suggestive to possible DNA intercalation. Compound 7 did not show these effects. The relative order of DNA binding was 3 > 2 > 4 > 5, which roughly followed the order of cytotoxicity of these compounds. Since the fluorometric method is primarily a qualitative presumptive method and the fluorescence and quenching may also result from nonintercalative DNA binding, other corroborative studies such as viscometric and affinity chromatography are needed to confirm and quantitate an intercalating mechanism. Detailed results of these DNA-binding studies are to be reported elsewhere. Nonetheless, there is a clear indication that the structurally simplified congeners of dercitin bind and/or interact with double-stranded nucleic acid.

Further spectrofluorometric studies were carried out using the synthetic oligonucleotides poly[dA-dT]·poly[dA-dT] and poly[dG-dC]·poly[dG-dC] in place of native DNA. In these preliminary studies the degree of fluorescence quenching was higher with the G-C polynucleotide than the A-T polynucleotide, with native calf-thymus DNA being intermediate, suggestive of a guanine-cytosine sequence selectivity in DNA binding. Such a preference for G-C base pair sequences is well-known for acridine-based intercalating agents[18] and appears to hold true for the 9-acridinone derivatives 4 and 5 as well. Only a low degree of binding to single-stranded *Escherichia coli* tRNA was observed. While studies have as yet to be completed, studying their intercalative behavior with HIV-nucleotide target sequences, it seems likely that compounds 4 and 5 may exert their antiviral actions in part through an intracellular DNA-binding mechanism. HIV-1 is a retrovirus whose genomic nucleic acid is RNA. However, in order to replicate, it must produce a DNA transcript of its genome using the enzyme reverse transcriptase. It is conceivable that intercalating agents could act on such a transcript and thus prevent retroviral replication, or could interact with the RNA–DNA double-stranded hybrid during the process of reverse transcription. Thus they may interfere with retroviral replication in a manner similar to the "antisense oligonucleotides" which interfere with the DNA–RNA interaction in reverse transcription.[19]

Agents with a G–C sequence selectivity of nucleic acid interaction and low cytotoxicity profiles towards mammalian cells such as 4 and 5 could prove useful antiviral agents in the treatment of HIV-1 infection. Certain segments of the HIV genome which have regulatory function and are common among different viral strains could be the targets in the design of such compounds. One of the major disadvantages seen with reverse transcriptase inhibitors, such as the TIBO derivatives[20] or nevirapine,[21] is that they

Table V. Tumor Cell Proliferation Inhibition by Analogues in Vitro

compound	compd concn (μM)	tumor inhibition: % survival MCF-7M breast	Colo 320DM colon	Calu3 lung
5-fluorouracil	0.7	29	79	80
	7.0	10	5	32
	70	5	2	17
2	0.015	91	117	98
	0.15	38	49	36
	1.5	0	0	0
4	0.6	85	110	113
	6.0	19	41	48
	60	0	0	0
5	0.6	91	107	104
	6.0	86	108	73
	60	44	87	44

are specific only in inhibiting HIV-1 and have no effect on HIV-2 replication. The *sor* (short open reading frame) fragment of the HIV-1 RNA genome is rich in G and C repeat segments and is common to all replication-competent HIV.[22] An intercalating agent which binds to its DNA transcript with G-C sequence selectivity and an affinity towards viral nucleic acid could thereby inhibit HIV replication and prove useful therapeutically.

While a compound which intercalates with viral nucleic acid may also be expected to interact with the host T-lymphocyte DNA, there are likely to be considerable qualitative/quantitative differences in these respective interactions which may impart a degree of selectivity of antiviral action. For example, mammalian genomic DNA is longer and supercoiled compared to HIV-1-transcribed DNA and this may be responsible for considerable differences in binding affinity of compounds towards viral and mammalian nucleic acids. There is, in fact, a precedence for such selectivity of interaction. Antimalarial drugs such as quinacrine, for example, have a selectivity of toxicity towards the *Plasmodium* parasite's nucleic acid.[23] Compounds 4 and 5 retain the tricyclic planar acridine structure required for intercalation, but lack the basic nitrogen atoms of the thiazole ring, pyridine ring, and the (dimethylamino)ethyl side chain of the prototype molecule dercitin. These nucleophilic groups may impart to the dercitin molecule certain cytotoxic activity towards mammalian cells unrelated to intercalation, by virtue of being capable of binding to acidic amino acid residues in cellular structural proteins and enzymes. These functional groups having been eliminated in 4 and 5; these compounds may exhibit lower cytotoxicity. Thus, cytotoxicity in dercitin congeners may be decreased by figuratively "trimming off the fat" from the natural product by sequential elimination of the basic nitrogen groups in its structure, to design compounds with higher antiviral therapeutic indices (Figure 1). Compounds 4 and 5 also possess a mercaptoacetic acid side chain which may help

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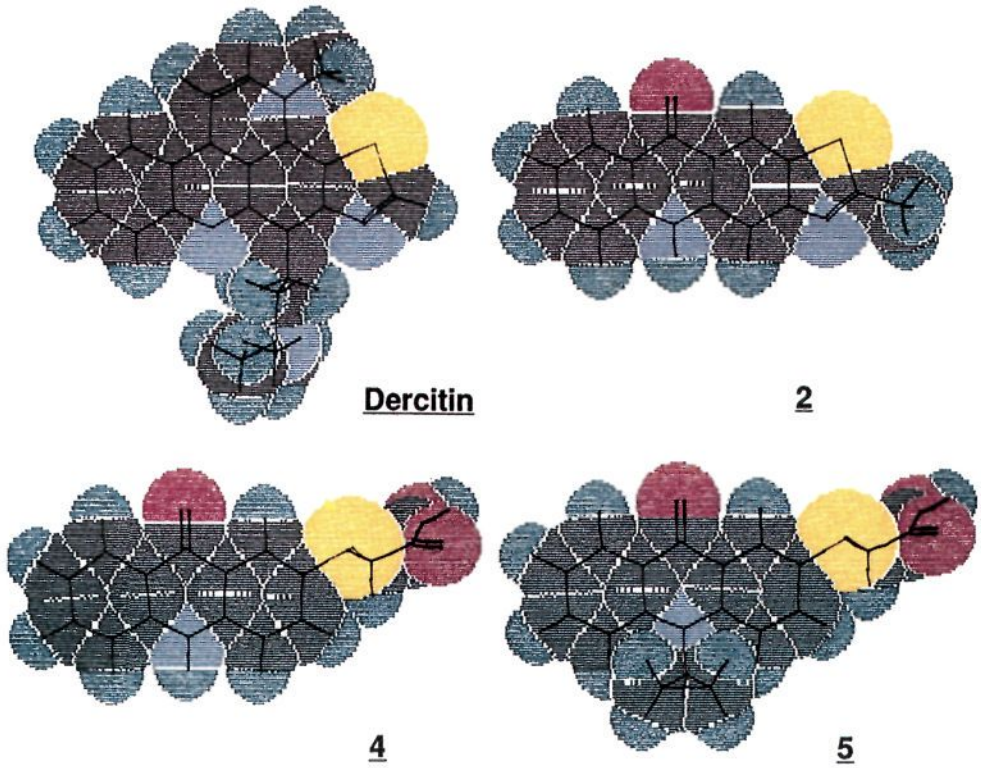

Figure 1. Structural comparison between dercitin and its simplified congeners.

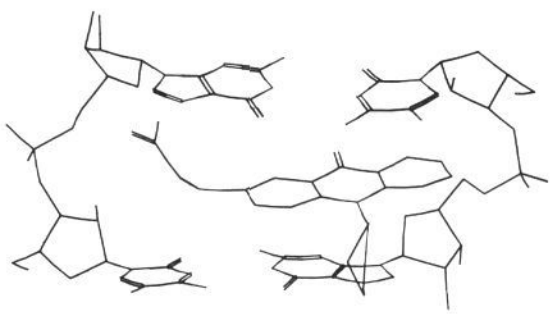

Figure 2. Mode of intercalation of **5** between G-C/C-G base-pairs in double-stranded DNA. The carboxyl group may serve as an anchor by an ionic interaction with the N-3 nitrogen of a purine base.

to anchor the molecule at the site of intercalation between base-pairs by a "salt-bridge" interaction with the N-3 atom of a flanking purine ring (Figure 2).

On the other hand, in the design of antitumor agents based on the structure of dercitin, it is desirable to retain a higher degree of cytotoxicity in the molecule. Evaluation of the analogues **2**, **4**, and **5** against the proliferation of breast, colon and lung tumor cell lines in vitro led to the results shown in Table V. It is clear from these data that the analogue **2**, which retains the tetracyclic thiazoloacridine ABCD ring system of dercitin, has the highest level of cytotoxicity against tumor cell lines, showing complete inhibition of all three tumor types at a concentration of 1.5 μM, comparable to that produced by a 5-fluorouracil (5-FU) control at 70 μM. Compound **4** retained a lower measure of tumor cell proliferation inhibition while **5** was considerably less effective. It is interesting that these analogues were cytotoxic to the tumor cells at 60 μM since they were not toxic to nonmalignant MT-4 cells up to 400 μg/mL (>1000 μM).

The following structure–activity relationships have resulted from the study of simplified congeners of dercitin:

(a) Progressive structural deletion of basic nitrogen atoms in the dercitin molecule leads to derivatives with lower cytotoxicity.

(b) The 9-acridinone derivatives retained the ability to bind DNA, but were less cytotoxic than acridines.

(c) The analogue **2**, which retains the thiazole ring of dercitin and is a linearly fused tetracyclic structure which closely approximates doxorubicin (adriamycin) and ellipticine in molecular shape and size (Chart I), had tumor cell proliferation inhibitory activity approaching that exhibited by the natural product[1,2] and was effective against all three tumor types evaluated. This suggests that the nonlinearly fused pyridine ring E in the structure of dercitin may not be necessary for antitumor effects of the molecule. However, the analogues **4** and **5** which had the best anti-HIV activity were less effective in tumor cell proliferation inhibition; suggesting that the fused thiazole ring contributes to the cytotoxicity of **2** and dercitin. Compound **2** is being further evaluated for antitumor activity.

(d) The sulfur atom in the dercitin molecule seems essential for antiviral activity; the imidazo[4,5-*b*]acridine **6** in which the sulfur atom is replaced by a nitrogen was not effective in neutralizing HIV-1.

(e) Analogues **4** and **5** with a mercaptoacetic acid group at the C-2 position of the tricyclic ABC acridine nucleus of dercitin showed the highest HIV-1 neutralization activity and also partially inhibited viral binding to lymphocytes, at noncytotoxic concentrations. They also retain the ability to bind DNA in vitro and may thus represent antiviral compounds with a dual extracellular/intracellular mode of action. An intercalative mechanism of action may further be indicated by the fact that the noncoplanar congener **7** does not inhibit HIV-1 replication in the MT-4 cell neutralization assay. The presence of the acidic carboxylic groups may contribute to the ability of **4** and **5** to prevent viral binding to lymphocytes; such activity has

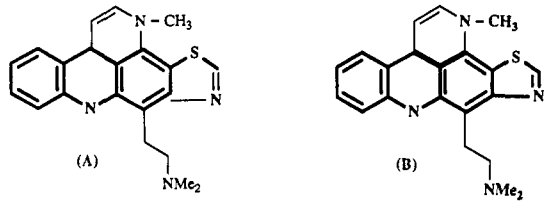

Figure 3. Bold outline indicates putative pharmacophores for favorable antiviral (A) and antitumor (B) activities in dercitin analogues.

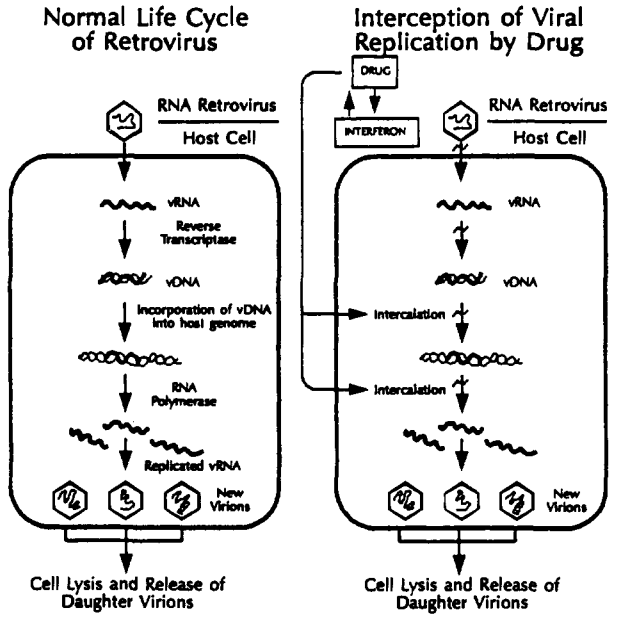

Figure 4. Modes of inhibition of retroviral replication by acridine-based antiviral agents.

been previously noted in aurincarboxylic acids.[24] and naphthalenesulfonic acids.[25]

In conclusion, the prototype molecule dercitin was used as a lead compound to isolate the structural features imparting antiviral and antitumor activities to the natural product. The putative pharmacophores for these activities are shown in Figure 3.

Compounds 4 and 5 represent a novel structural type effective at HIV-1 infectivity neutralization in MT-4 lymphocytes at noncytotoxic doses. While their potency levels are considerably lower than that of nucleoside-type agents such as AZT, the micromolar levels of activity seen compare with those seen for naphthalenesulfonic acids[25] and anthraquinones,[26] non-nucleoside compounds previously reported to inhibit HIV-1 replication in vitro. Aminoacridine derivatives have also been previously shown to inhibit retroviral replication.[27] The variation in activity of test compounds against different type of leukocyte cell lines noted in this study have also been observed by others.[25] Further analogues with the 9-acridinone-2-mercaptoacetic acid substructure are being prepared with a view towards improving potency within the series as well as increasing lipophilicity and ability to penetrate the blood–brain barrier. It is worth noting that while compound 5 resembles the recently described anti-HIV agent nevirapine[21] in being a tricyclic structure with a *N*-cyclopropyl group, it appears to have a different mechanism of action. Nevirapine is not a coplanar structure and has a puckered butterflylike conformation about the central ring, incapable of DNA intercalation. Nevirapine is a potent reverse transcriptase inhibitor,[21] a mode of action not seen with 4 and 5. Newer non-nucleoside compounds which inhibit HIV-1 replication need to be identified which have alternate mechanisms of action to target virus in the brain[28] and circumvent the side effects and the drug resistance to AZT that have been observed on long-term therapy.[29]

The results of these studies indicate that novel antiviral/antitumor agents may be designed using elements of the structure of the lead molecule dercitin and the structurally related marine alkaloids. Optimized cytotoxicity profiles may be derived by appropriate structural modification of the identified pharmacophores, leading to compounds suitable as selective antiviral or antitumor agents.

Experimental Section

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian 390, 90-MHz instrument, and the chemical shifts are reported in δ (ppm) values downfield from a tetramethysilane internal standard. Elemental analyses were determined by Microanalysis, Inc., Indianapolis, IN. Fluorescence studies with DNA were carried out using a Turner R112 digital fluorometer. Viral binding to H-9 lymphocytes was determined using a Becton-Dickinson FACS-SCAN instrument. γ-Interferon production was assayed using a commercial RIA kit from Centocor, Malvern, PA. Calf thymus DNA, *Micrococcus lysodeikticus* DNA, *E-coli* tRNA, and (poly dA·poly dT)·(poly dA·poly dT) were obtained from Sigma Chemical Co. (St. Louis, MO), and (poly dG·poly dC)·(poly dG·poly dC) was obtained from Pharmacia, Uppsala, Sweden. Molecular modeling studies used the Alchemy II software program (Tripos Associates, St. Louis, MO).

The synthesis of 2-methyl-5,10-dihydrothiazolo[5,4-*b*]acridin-10-one, 2, 2-methylthiazolo[5,4-*b*]acridine, 3, and 2-[(2-methyl-5-benzothiazolyl)amino]benzoic acid, 7, have been recently reported by Taraporewala.[13]

[(9,10-Dihydro-9-oxo-2-acridinyl)thio]acetic acid, 4, was prepared as previously described by Taraporewala and Kauffman.[7]

[(10-Cyclopropyl-9,10-dihydro-9-oxo-2-acridinyl)thio]acetic Acid (5). To a solution of 2.75 g (0.01 mole) of 4 in methanol (30 mL) was added a solution of diazomethane in ether (30 mL) at 0 °C. The mixture was stirred for 30 min and the solvent was removed in vacuo. The residue was dried overnight in a vacuum oven (20 mmHg, 60 °C) to yield 2.89 g of crude methyl ester. The ester so obtained was dissolved in 20 mL of dry *N*,*N*-dimethylformamide; sodium hydride (560 mg of a 60% dispersion in mineral oil, washed with hexanes, 0.025 mol) was added, and the mixture was heated at 60 °C for 2 h. A highly UV-fluorescent solution resulted. The reaction mixture was cooled to room temperature, and a solution of bromocyclopropane (1.452 g, 0.012

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mol) in dry *N,N*-dimethylformamide (5 mL) was added dropwise. The reaction mixture was heated at 65 °C for an additional 2.5 h, then cooled to room temperature, and poured into ice–water. The yellow precipitate was filtered; the filter cake was washed with water and the still moist residue of the *N*-cyclopropyl methyl ester was dissolved in 30 mL of methanol. A solution of NaOH pellets (1.2 g) in 10 mL of deionized water was added, and the mixture was warmed to 65 °C for 1 h. The filtrate was diluted with 100 mL of deionized water, and 6 N HCl was added to precipitate a golden-yellow solid. This was filtered, washed well with water, and dried. The residue (2.94 g) of crude product was applied to an Ace-Kauffman chromatographic column packed with alumina and eluted using methanol. The eluate was evaporated, and the residue was recrystallized from aqueous methanol to yield 2.03 g (64.1%) of **5**; mp 200–202 °C dec; ^{1}H-NMR (DMSO-d_6) δ 0.85–1.08 (m, 4 H, cyclopropyl CH_2CH_2), 3.8 (m, 1 H, cyclopropyl methine), 3.86 (s, 1 H, SCH_2), 7.08–8.25 (m, 7 H, arom H). Anal. $C_{18}H_{15}NO_3S$ (C, H, N, S).

2-Amino-5,10-dihydro-10-oxoimidazo[4,5-*b*]acridine Hydrobromide (6). To a solution of 2,3-diamino-9,10-dihydro-9-oxoacridine (1.4 g) in dry dichloromethane (20 mL) was added cyanogen bromide (30 mL of a 3.0 M solution in dichloromethane). The mixture was stirred in a fume hood for 72 h at room temperature; the dichloromethane solvent was removed in a stream of nitrogen. The residue was triturated with acetone, filtered, washed with cold acetone, and dried to yield 1.36 g of a yellow-green solid: mp 290–294 °C dec; ^{1}H-NMR (DMSO-d_6 + CD_3OD) δ 5.82 (bs, 3 H, NH_2, NH), 6.96–8.24 (m, 6 H, arom H). Anal. $C_{14}H_{11}N_4BrO$ (C, H, N).

DNA-Binding Study. This was performed by a fluorometric method, essentially by the procedure of Fikus et al.[17] Briefly, DNA solutions of calf thymus DNA, *M. lysodeikticus* DNA, or synthetic oligonucleotides were prepared in 0.1 M sodium acetate buffer (pH 5) at a DNA concentration of ~1.5 μM in base-pair concentration. The DNA solution was treated with ethidium bromide (2.6 μM) and varying concentrations of test compounds (nine replicates) ranging from 10 to 500 μM, and the solutions were equilibrated at 25 ± 3 °C for 24 h. A negative control contained no test compound. Fluorescence intensities at varying compound concentrations were measured using a 610-nm filer, a concentration-dependent variation in fluorescence intensity indicated DNA binding by the test compound. The lower the drug concentration required to produce a given change in fluorescence intensity, the higher its affinity for nucleic acid binding.

Cytotoxicity Testing in MT-4 Lymphocytes. MT-4 cells (5×10^4 cells/well) were incubated in a 24-well plate and cultured in the presence of various doses of test compounds and in their absence. Compound solutions were diluted in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS), 10 mM HEPES buffer, 50 μg/mL of penicillin, 50 μg/mL streptomycin, and 2 mM glutamine and then sterile-filtered. The total incubation volume was 1 mL. On day 4 of incubation, cells were resuspended, and a 20 μL aliquot was removed to study cell viability using the trypan blue exclusion method; the identical procedure was repeated on day 7. Incubations were carried out at 37 °C using a 5% CO_2 atmosphere. All incubations were performed in triplicate. Results are expressed as % viable cells (Table I).

HIV-1 Infectivity Neutralization Assay. Compounds at specified concentrations (Table I) were added to 100 μL of HIV-1 (Molt IIIB strain) suspension containing ~2×10^4 tissue culture infective dose ($TCID_{50}$) for 1 h at 37 °C in an incubator (5% CO_2 atm). After incubation, the treated virus was added to MT-4 cells at a density of 5×10^5 cells/well; the final volume per well was 1 mL. Treated virus and cells were placed in 24-well culture plates and incubated at 37 °C in an incubator (5% CO_2 atm). Viability counts were performed on days 4 and 7 of incubation using the trypan blue exclusion dye method. The positive control consisted of MT-4 cells plus virus suspension, and the negative control was MT-4 cells plus culture medium. Triplicate samples were used at each concentration. Results are expressed as % viable cells (Tables I and II).

HIV-1 Inactivation by Microtiter Tetrazolium Salt (MTT) Assay. The rapid colorimetric assay for cell growth and survival initially described by Mossmann[30] and subsequently applied by HIV-1-infected MT-4 cells by Robertson et al.[31] was adopted. Test compounds at specified concentrations (50-μL solution) were added to wells in a 96-well cell culture plate. A 50-μL inoculum of each of HIV-1 (Molt IIIB) or HIV-MN strains of virus in RPMI 1640 medium was added following a 1-h incubation at room temperature. The compound–virus mixture was then added to 100 μL of culture medium (RPMI-1640) containing 5 ~ 10^4 MT-4 cells and incubated at 37 °C in a humidified atmosphere at 5.0% CO_2. Cell viability was assayed at 7 days by adding 10 μL of a solution of the tetrazolium salt MTT (Sigma Chemical) in PBS-saline buffer at 5.0 mg/mL to the wells and incubation at 37 °C for 4 h. The dye uptake was stopped by adding 200 μL/well of 0.04 N HCl in 2-propanol. The resulting color intensities were quantitated by optical density (OD) measurements using an automated ELISA plate reader with a 570-nm filter. Percent inactivation produced by compounds was determined by the following formula: Results are indicated in Table II. Percent

$$\% \text{ inactivation} = \left[1 - \frac{(\text{OD MT-4}) - (\text{OD compound-treated virus})}{(\text{OD MT-4}) - (\text{OD untreated virus})}\right] \times 100$$

inactivation reported is the mean of three samples at each concentration.

Inhibition of HIV-1 Binding to H-9 Lymphocytes. An immunofluorescent flow cytometric technique[15] was adapted to study the inhibition of HIV-1 to uninfected H-9 cells produced by test compounds. An aliquot of 50 μL of Molt IIIB strain of HIV-1 was incubated with test compounds overnight at specified concentrations (Table II). The following day the treated virus, as well as a control sample of virus incubated in culture medium overnight, was incubated with uninfected H-9 cells at 37 °C for 2 h in an incubator (5% CO_2 atm). After incubation, the cells were washed with 2 mL of PBS–5% FBS–0.01% NaN_3 and then incubated with a 1:100 dilution of an HIV-positive human serum for 30 min at 4 °C. The cells were washed three times with PBS–FBS–NaN_3 and a 1:10 dilution of goat antihuman IgG conjugated to fluorescein isothiocyanate (FITC) for 30 min at 4 °C in the dark. The cells were then washed once with 2 mL of PBS-buffer and fixed with a 0.5% methanol-free formaldehyde solution. Samples were held at 4 °C in the dark prior to analysis on a Becton Dickinson FACS SCAN instrument. Results are expressed as % inhibition of binding of virus to lymphocytes (Table III).

γ-Interferon Assay. Test doses of compounds, in triplicate, ranging from 100–400 μg/mL, were incubated with MT-4 lymphocytes at a cell density of 5×10^4 cells/well in a 96-well cell culture plate overnight at 37 °C. After incubation, supernatant was collected and stored at −80 °C until analysis. Analysis for γ-interferon was carried out using an anti-IFN-γ ^{125}I-labeled monoclonal antibody kit from Centocor (Malvern, PA) by the procedure recommended by the vendor. Positive controls used were the mitogens concanavalin A and pokeweed mitogen. None of the test compounds induced significant levels of γ-interferon; significant induction was observed by the control mitogens.

Cytotoxicity and HIV-1 Inhibition in Human PBMC. This was studied using the procedure previously reported by Schinazi et al.[26] Briefly mitogen-stimulated human PBMC (10^6 cells/mL) were infected with 100 $TCID_{50}$/mL of HIV-1 (strain LAV) and cultured in the presence and absence of various concentrations of test compounds, drugs being added 45 min post-infection. Five days subsequent to infection, the cultures were studied for cell viability by the trypan blue dye exclusion method. The drugs were also evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells using a radioactive thymidine uptake method. Briefly, cells in a 96-well plate were grown in the presence of drug for 24 h, and then 1 μCi of [^{3}H]thymidine was added to each well. After 24 h, the cells were harvested on

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glass fibers, washed, and dried, and the amount of radioactivity associated with the cells was determined. Cycloheximide, a potent protein inhibitor, was included as a control for toxicity in every assay. Results are expressed as EC_{50} for viral inhibition and IC_{50} for cytotoxicity in μM (Table III).

Inhibition of Tumor Cell Proliferation in Vitro. Antitumor evaluation of compounds **2**, **4**, and **5** was carried out against breast, colon, and lung tumor cell lines. Specified concentrations of test compounds were added to cell cultures of the tumor cells in RPMI-1640 medium in 1% agar supplemented with 5% fetal bovine serum and incubated for 1 week. At the end of the incubation period, cell viability was assessed in comparison with a control sample in which distilled water replaced the drug solution. A positive control of 5-fluorouracil was used as a standard cytotoxic drug. Results are expressed as the concentration-dependent cell survival (Table IV).

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Note Added in Proof: Since the acceptance of this paper, a structural revision for the *Dercitus* alkaloids has been published: Gunawardana, G. P.; Koehn, F. E.; Lee, A. Y.; Clardy, J.; He, H-Y.; Faulkner, D. J. Pyridoacridine Alkaloids from Deep-Water Marine Sponges of the Family Pachastrellidae: Structure Revision of Dercitin and Related Compounds and Correlation with the Kuanoniamines. *J. Org. Chem.* **1992**, *57*, 1523–1526. The revised structures contain a [4,5-*b*]thiazole regiochemistry similar to that of Kuanoniamine A shown in Chart I.