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Review Article

Plant photosensitizers with antiviral properties

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Summary

Many antiviral compounds obtained from plants are photosensitizers, i.e., their biological properties are dependent upon or augmented by light of specific wavelengths, commonly long wave ultraviolet, UVA. Three groups of chemically distinct plant photosensitizers have been investigated in some detail in regard to antiviral properties. These are (a) thiophenes and polyacetylenes; (b) furyl compounds; (c) certain alkaloids. Some of the thiophenes and their acetylenic derivatives possess extremely potent phototoxic activities toward membrane-containing viruses. These activities are markedly affected by the chemical structures of these compounds. Inactivated virus retains its integrity, however, and penetrates cells, but does not replicate. Their mechanism of action is believed to occur via singlet-oxygen damage to the membranes, although other targets cannot be ruled out.

In contrast, the antiviral activities of plant furyl compounds (such as psoralens and furanochromones) appear to depend on UVA-mediated covalent adduct formation with the viral genomes.

Some of the photoactive β-carboline alkaloids also have impressive antiviral activities, especially against viruses with single-stranded genomes. These and other types of alkaloids appear to work by mechanisms that do not require covalent bonding to nucleic acids, and may also involve other target molecules as well.

Some of these compounds have potent antiviral activities at concentrations well below cytotoxic levels, and accordingly should be tested in animal models.

Photosensitizer; Phytochemical; Thiophene; Polyacetylene; Furyl compound; Furocoumarin; Furanochromone; Alkaloid; β-carboline.

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Introduction

Many plants throughout the world, including some with documented medicinal properties, contain chemicals that are toxic to micro-organisms. In many cases this biological activity is strictly dependent on, or is enhanced by, light of specific wavelengths, such as long wave ultraviolet radiation UVA (320–400 nm) (e.g., see action spectrum for α-terthienyl in Fig. 1). The ultraviolet spectrum is divided into discrete segments referred to as UVC, UVB, and UVA, from shorter to longer wavelengths. Each segment is associated with distinct biological effects (Spikes, 1977). The UVA is particularly prevalent in sunlight, and passes readily through transparent glass and plastic materials. Many of the compounds that we have studied for antiviral activity require UVA.

Essentially a photosensitizer is a chemical which can be excited to a new electronic state by the absorption of a photon and which, in the excited state, reacts with itself or a second type of molecule. In biological systems this second molecule is frequently oxygen, which is thereby converted to a reactive oxygen species, such as singlet-oxygen ($^1O_2$), which then reacts with a nearby target molecule, such as the various membrane components or macromolecules of a micro-organism, in the so called photodynamic reaction (Spikes, 1977; Towers, 1984). The result is usually damage to the target molecule and hence the organism. The precise chemical details and the nature of the damage involving micro-organisms has seldom been elucidated.

Several years ago we undertook to examine representatives of different types of known plant photosensitizers for possible antiviral activities, with a view to understanding their mechanisms of action against relatively simple organisms, and to determine their potential as useful antiviral compounds. These categories are (a) thiophenes and polyacetylenes; (b) furyl compounds; (c) β-carbolines and other alkaloids.

Only certain furyl compounds (psoralens) had previously been studied for antiviral activity (see below). In addition, several alkaloids unrelated to carbolines had also been evaluated, but not in connection with a role for light (Vanden Berghe et al., 1986; Hudson, 1989).

In order to investigate these compounds as photosensitizers, virus and compound are incubated, in plastic trays or tubes that are impermeable to UVB and UVC, while exposed to radiation from a bank of so-called black-light blue (BLB) lamps under standard conditions. The emitted light ranges from 320–400 nm, with a peak at 350 nm. Residual infectious virus may then be titrated on cell cultures in the dark. Various control tests are also carried out simultaneously (Hudson and Towers, 1988; Hudson et al., 1985). If desired, virus-infected cells, or cells prior to infection, can also be exposed to UVA in the presence of the compound. However all the compounds described here are virucidal.

Polyacetylenes and thiophenes

More than 700 polyacetylenes (polyines) have been characterized, and they are particularly prominent in the Compositae (Asteraceae), Umbelliferae (Apiaceae) and Campanulaceae, including many medicinal plants from various parts of the world. Their chemistry and distribution were described in detail by Bohlmann et al. (1973), while more recent reviews have focused on specific aspects of them (Towers, 1980; 1984; Hansen and Boll, 1986; Lam, Breteler and Arnason, 1988). Other polyacetylenes have also been found in algae, fungi, sponges, nudibranchs, sea hares and insects (Towers, 1984). They occur principally as straight chain polyines, allenes, phenyl and thiophenyl derivatives, thioethers and spiroketalenol-ethers (e.g., see Fig. 2). They are often found in the form of acids, alcohols, esters, aldehydes and chlorides of the corresponding C_8–C_{18} hydrocarbons (Ferreira and Gottlieb, 1982).

The concentration of polyacetylenes in plants is sometimes quite high: e.g., up to 1% of the fresh weight in species of Compositae, although their distribution between roots, leaves, stems, flowers and seeds is quite variable and seasonal. Their function in the plants, if any, is not known, although they have been implicated in defence mechanisms against insect and fungal pests.

A particularly interesting, yet puzzling, observation is that many, but not all, of these polyacetylenes are UVA requiring photosensitizers. Earlier observations recorded the phototoxicity of many extracts of the roots of Compositae toward nematodes, insect larvae, fungi and some bacteria, and eventually those activities were ascribed to UV-absorbing polyacetylenes or thiophenes (Towers, 1984). The purified compounds elicit their effects rapidly, and probably through a $^1O_2$ mediated mechanism, although there is some controversy on this point (Towers, 1984; Kagan et al., 1984; Kagan, 1987; Arnason, 1988; Gong et al., 1988).

The thiophenes (Figs. 1 and 2) and related sulfur-containing compounds such as

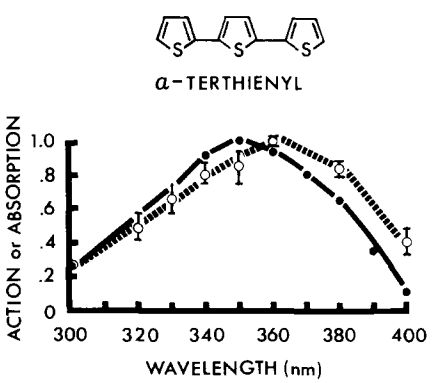
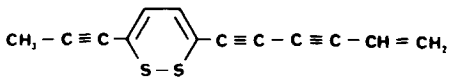
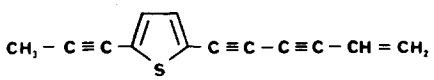


Fig. 1. Action spectrum for α-terthienyl, showing the approximate coincidence of the UV absorption spectrum with the biocidal activity (in this case photo-killing of mosquito larvae; courtesy of Dr. G.H.N. Towers). The BLB (UVA) lamps used in the antiviral tests have a peak emittance of approximately 350 nm.

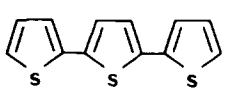


THIARUBRINE-A

(T-A)

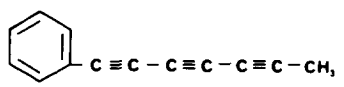


THIOPHENE-A



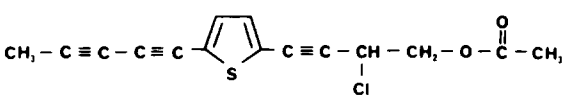
α-TERTHIENYL

(α-T)



PHENYLHEPTATRIYNE

(PHT)



ACBP-THIOPHENE

(ACBP-T)

Fig. 2. Structural formulae for thiophenes and polyacetylenes tested for antiviral activity.

dithiacyclohexadienes (e.g. thiarubrine A), are usually grouped together with the polyacetylenes because of their common biosynthetic pathways. Many of these thiophenes are also widely distributed in the plant kingdom and are often photosensitizers in the UVA region. Like the polyacetylenes, some of them are phototoxic to a variety of organisms, although some of their biological activities do not require light (Towers et al., 1985; Constabel and Towers, 1989). A particularly interesting feature of some of the thiophenes is their high potency, their photo-

toxicity being noticeable frequently at concentrations which are orders of magnitude lower than polyacetylenes. This has made them attractive candidates for large scale uses such as insecticides (Arnason et al., 1987).

The mechanism of action of thiophenes is better understood than it is for polyacetylenes. Thus thiophenes such as α-terthienyl exert their phototoxicity on lipid components of membranes via $^1O_2$ (McRae et al., 1985), although other potential targets and mechanisms may exist (Downum et al., 1983; Hudson and Towers, 1988a,b).

Antiviral properties of polyacetylenes and thiophenes

Our initial studies were done with phenylheptatriyne (PHT; Fig. 2), which we determined was phototoxic to viruses with membranes: murine CMV, Sindbis virus, the salmonid rhabdovirus IHNV (infectious hematopoietic necrosis virus), and bacteriophage PM2; but was not active against phage T4 or the fish virus IPNV (infectious pancreatic necrosis virus), which do not possess membranes (Warren et al., 1980; Hudson et al., 1982).

Further analysis revealed that when MCMV, which had been inactivated by exposure to PHT and UVA, was added to mouse cells, the virus penetrated normally and the viral DNA entered the nucleus, the normal site of replication. Although the virus particles, and the viral DNA itself, retained their integrity, viral genes were not expressed and the virus thus failed to replicate (Hudson et al., 1986a).

We extended these studies to include α-terthienyl (αT), thiarubrine and the other compounds shown in Fig. 2. Each compound was tested for its effect on the viruses, MCMV, SV, T4 and M13 in the presence and absence of UVA. Based on the data obtained from virus-survival curves such as those shown in Fig. 3 (Hudson et al., 1986b,c; 1987), we constructed LD_{99} values to compare the relative potencies of the compounds. An example is shown in Table 1. The principal conclusions from these studies were: (a) α-terthienyl is by far the most potent antiviral compound in the group, and in fact it displayed significant phototoxicity against MCMV and SV at 10^{-5} μg/ml ($\sim 3 \times 10^{-11}$ M); (b) not all compounds were antiviral; thus thiophene A (Figs. 2,4), the photolysis product of thiarubrine A, was only slightly active at 10 μg/ml; (c) none of the compounds had antiviral activity in the dark; (d) the minimal effects seen with the bacteriophages required much higher concentrations of the compounds than the anti-MCMV and anti-SV effects. This probably reflects two distinct mechanisms of activity, involving membranes, when present or viral proteins or nucleic acids; (e) cytotoxic effects were seen only at concentrations much higher than the antiviral effects. This is especially so of α-terthienyl which did not show phototoxicity to mouse 3T3 cells below 0.005 μg/ml. Thiarubrine A became phototoxic to cells at > 0.1 μg/ml and at 10 μg/ml it was also cytotoxic in the dark.

Thus in general the therapeutic index for these compounds, especially α-terthienyl and thiarubrine A, appears to be quite high. In support of this belief it was found that mice and rats could tolerate injections of > 5 mg/kg body weight of

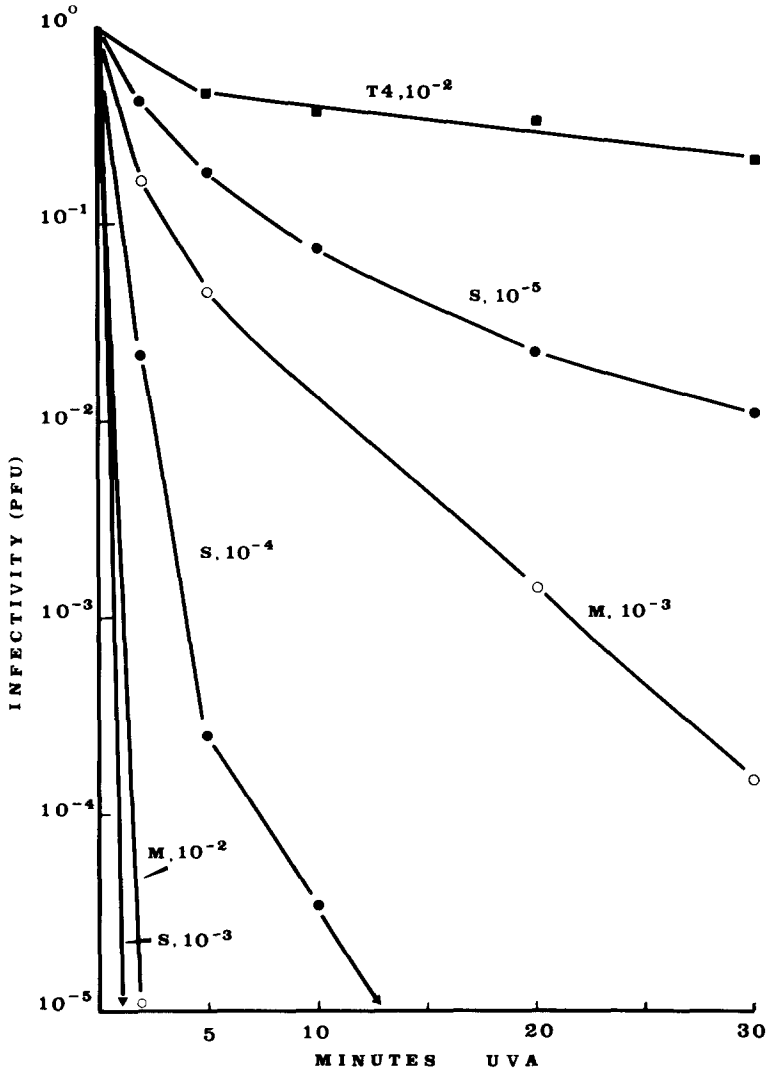


Fig. 3. Virus survival curves for Sindbis virus (S), murine cytomegalovirus (MCMV), and phage T4, treated with α-terthienyl at the concentrations indicated (μg/ml), and varying times of UVA radiation. In the absence of UVA, all virus titers remained at the original level of 1×10^8 PFU/ml. (From Hudson et al., 1986b.)

these compounds, in the absence of deliberate UVA exposure. Furthermore, chimpanzees in the wild have been observed to consume daily large numbers of *Aspilia* leaves which are rich in thiarubrine A (Rodriguez et al., 1985).

When cells were infected with α-terthienyl + UVA-inactivated MCMV, the virus retained its integrity (as seen in the electron microscope) and it penetrated readily into cell nuclei; but no viral gene expression was detected. Thus polyacryl-

TABLE 1

Relative antiviral phototoxicity of thiophenes and polyacetylenes

Compound[a]	LD_{99} for[b]		
	SV	MCMV	T4
α-terthienyl	30	194	12000
thiarubrine-A	1140	625	~17500
phenylheptatriyne	3168	6272	NDA
ACBP-thiophene	NT	5812	NT
thiophene-A	>>12000	>>12000	NDA

[a]Concentration of 0.1 μg/ml. Each compound was incubated (at 4°C) with 10^8 PFU of virus while exposed to UVA. After various times of exposure, from one to 30 min, samples were removed, and kept in the dark until all samples had been collected. They were serially diluted for plaque-assays. The LD_{99} values were derived from the kinetic survival curves (such as those shown in Fig. 3).
[b]LD_{99} = dose of radiation (seconds of exposure × 5 watts/m^2) required to decrease infectivity by 99%. NT, not tested; NDA, no detectable activity. (Data from Hudson et al., 1986b, and unpublished results).

amide gel electrophoresis demonstrated the absence of viral protein synthesis, and nucleic-acid hybridization tests failed to show synthesis of viral RNA or viral DNA (Hudson et al., 1986b).

We have more recently evaluated a series of thiophenes, synthesized by Dr. R. Rossi and colleagues at the University of Pisa (Carpita et al., 1985a,b; Rossi, 1988). Some of these compounds also occur in plants. A total of 31 compounds was tested (numbered 1–31), together with α-terthienyl as a reference against which relative potency could be assessed.

In order to relate the antiviral properties quantitatively, we devised a method to assess several compounds simultaneously and over a wide range of concentrations. In essence this test measured the minimum concentration of a compound which could completely inactivate a given number of infectious virus particles (10^4 PFU of SV or MCMV) following a standard dose of UVA radiation. The results represent a direct measure of the relative potency of a compound. These MICs (minimum inhibitory concentrations) are presented in Table 2, for the most active

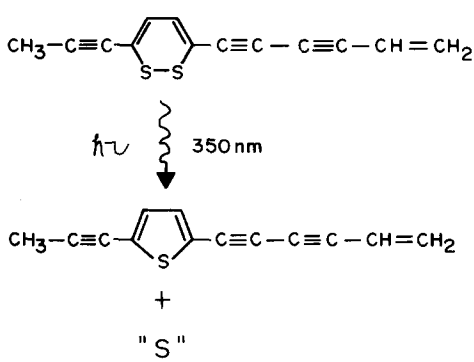
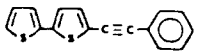
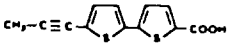
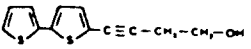
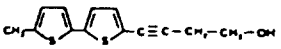
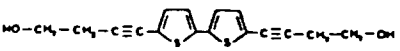
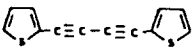
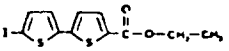


Fig. 4. Photolysis of thiarubrine-A to thiophene-A by UVA.

TABLE 2

Summary of potent antiviral thiophenes

Compound no.	Formula	MIC[a] vs SV (μg/ml)	Potency[b]	MIC[a] vs MCMV (μg/ml)	Potency[b]
αT		0.07	1.00	0.062	1.00
1		0.07	1.00	0.016	3.87
2		0.164	0.43	0.004	15.5
10		0.256	0.27	0.055	1.13
12		0.102	0.69	0.021	2.95
13		0.562	0.12	0.003	20.7
14		0.062	1.13	0.047	1.32
18		0.141	0.50	0.062	1.00
23		0.027	2.60	0.062	1.00
27		0.001	70.0	0.035	1.78

[a]MIC, minimum concentration required to inhibit 10^4 PFU of SV or MCMV.
[b]Potency, MIC (αT)/MIC (Compound).

compounds, along with their relative potencies in comparison to α-terthienyl (arbitrarily set at 1.0). All of these compounds were tested simultaneously against the viruses in the dark, but none of them showed any significant activity, at a concentration of up to 1.0 μg/ml.

The compounds showed a wide range of antiviral activity, including some that were more potent than α-terthienyl and some that were completely inactive. In addition, the spectrum of relative potency was somewhat different for the two viruses, although in general the more potent compounds were active against both viruses.

Four compounds – numbers 1, 14, 23, and 27 – were equal to or greater than α-terthienyl in their activity against Sindbis virus (Table 2). These same compounds,

together with numbers 2, 10, 12, 13 and 18 were also equally potent or more potent against MCMV. These latter 5 were also moderately active against Sindbis, so that in general there was fairly good agreement between the results for the two viruses. The potencies shown in Table 2 are based upon μg/ml concentrations, although on a molar basis these values were similar. For simplicity, the structural formulae for the other compounds have been omitted; they are described in detail elsewhere (Hudson and Towers, 1988b).

Compound 27 was by far the most potent against Sindbis, whereas compound numbers 2 and 13 were the exceptional anti-MCMV compounds. The relative activities were not necessarily the same for both target viruses, however, possibly because their membranes are significantly different. Thus MCMV derives its membrane from the nuclear membrane of the host cell, whereas the SV membrane is derived from cytoplasmic membranes.

The common structural features of these very active compounds are the possession of two or three thiophene rings, at least one acetylenic substituent, conservation of conjugation, and a linear configuration. Other compounds (not shown) that did not have all these features, were much less active, or inactive. The presence of phenyl groups also tended to lower antiviral activity. This was due to relatively poor absorption of light in the UVA range, hence such compounds would not be sufficiently excited by our standard irradiation treatment. But other factors, such as relative quantum yields of singlet oxygen, were also important. The presence of halide groups did not necessarily preclude activity, e.g. the iodo-compound number 23. Some of these compounds were also cytotoxic in the presence of UVA (only); but there were very large differences between minimal cytotoxic concentrations and effective antiviral concentrations (unpublished data).

We are continuing these investigations on existing and additional thiophenes and polyacetylenes, since we believe that some of them may have therapeutic potential. It should be noted however that some of those compounds that were designated relatively weak or inactive under the test conditions may in fact possess a significant amount of antiviral photoactivity. The experimental procedure used for the evaluation of MICs in Table 2 was quite stringent, since the compound had to completely inactivate 10^4 PFU of virus. Nevertheless, the principal objective was to find compounds that were as potent, or more so, than α-terthienyl since these could be expected to represent more promising candidates for chemotherapy in vivo.

The mechanisms of action of two of these compounds, α-terthienyl and PHT, have been examined to some extent in various eukaryotic systems. The former appears to work on membrane constituents, possibly fatty acids, by means of an O_2-dependent mechanism (Towers, 1984; McRae et al., 1985). The mechanism of action of PHT is less clear, although again membranes appear to be targets.

We have also shown that both α-terthienyl and thiarubrine-A are slightly phototoxic to the bacterial virus T4, which does not possess a membrane (Hudson et al., 1986b,c).

These data together suggest that in addition to lipids some viral proteins may be targets for these compounds, although the mechanism by which such target-di-

rected photoactivity is converted into a block in the viral replication cycle is not understood.

Furyl Compounds

Furocoumarins (Fig. 5) are common constituents of many members of the Rutaceae and Umbelliferae (Apiaceae), although they have also been found in several other families of plants, and in fact are important components of a number of known medicinal plants. They can occur in concentrations of more than 1% by dry weight in various tissues of the plant. Biosynthetically they are derived from phenylalanine (Towers, 1984).

Their multiple biological activities have for some time been inextricably bound to their known photoactive attributes. Thus they are commonly phototoxic to cells, bacteria, fungi and viruses; the underlying cause usually being attributed to their capacity to intercalate in DNA followed by light-activated production of monoadducts or biadducts with pyrimidine bases, especially thymidine, although other mechanisms can also operate (reviewed in Song and Tapley, 1979; Hearst, 1981; Cimino et al., 1985). Some of them have been used therapeutically in the treatment of skin disorders such as psoriasis and vitiligo (the so-called PUVA therapy); in therapy of certain T-cell lymphomas (Edelson et al., 1987); and in other supposedly beneficial applications such as sun-tan creams and lotions, in spite of the

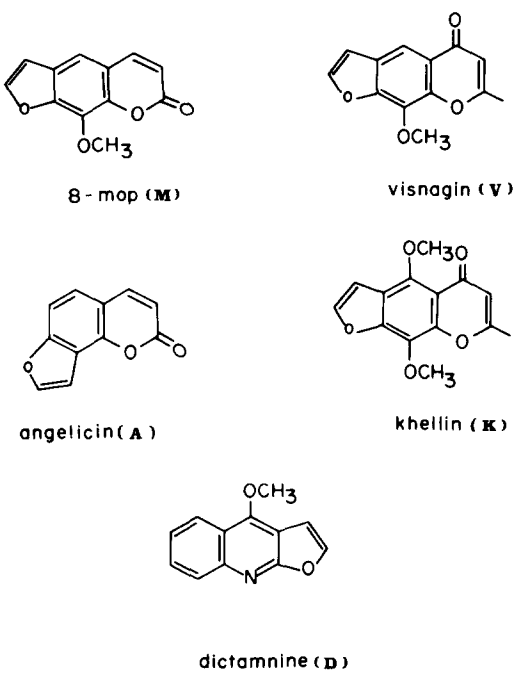


Fig. 5. Structural formulae of furyl compounds tested for antiviral activity.

suspected carcinogenic risks (Grekin and Epstein, 1981; Ashwood-Smith et al., 1982). In addition, plants containing furocoumarins are thought to be responsible for a significant number of photodermatoses in livestock, when the animals consume foliage containing such compounds and are subsequently exposed to sunlight (Ivie, 1982).

The furanochromones visnagin and khellin (Fig. 5) often occur in association with the coumarins, e.g. in species of *Ammi* (Umbelliferae). Extracts of these plants and their purified compounds have long standing medicinal applications (Abdel-Fattah et al., 1982). They are also phototoxic to cells, bacteria and fungi, and the similarity of their structures to the furocoumarins has led to the belief that their activity is manifest by a similar mechanism (Cassuto et al., 1977; Abysekera et al., 1983; Altamirano et al., 1985).

Antiviral properties of furyl compounds

The antiviral properties of furocoumarins, such as the psoralen derivatives (Fig. 5) have been studied by a number of investigators. Generally both DNA and RNA viruses, with or without membranes, were found to be susceptible, DNA viruses more so. These activities required UVA, although some reports have described low level antiviral effects in the dark (Hearst and Thiry, 1977; Hanson et al., 1978; Redfield et al., 1981), and the mechanism has usually been attributed to adduct formation between compound and viral nucleic acid, such that transcription was blocked (Song and Tapley, 1979; Cimino et al., 1985).

Among the initial group of furyl compounds we examined were the well studied 8-methoxypsoralen (8-MOP), the angular furocoumarin, angelicin; the furanochromones visnagin and khellin; and the furanoquinoline dictamnine. 8-MOP was the most potent in terms of antiviral activity against the DNA-containing murine cytomegalovirus (MCMV), while dictamnine and visnagin were moderately phototoxic to MCMV; but angelicin and khellin had very little activity. None of them were active in the absence of UVA. 8-MOP, visnagin and dictamnine were also somewhat phototoxic to the RNA-virus Sindbis (SV), as well as the bacterio-

TABLE 3

Summary of antiviral phototoxicity of furyl compounds

Compound	Relative activity against			
	T4 ds DNA −membrane	M13 ss DNA −membrane	MCMV ds DNA +membrane	SV ss RNA +membrane
8-MOP	+++[a]	+++	+++	+
Angelicin	++	+++	+	+
Dictamnine[b]	++	+	++	+
Visnagin	++	+	++	+
Khellin	+	+	+	±

[a]Number of + signs indicates relative degree of phototoxicity.
[b]Further discussion of dictamnine in section on alkaloids.

phages T4 and M13 (Hudson et al., 1985, 1988a). These results are summarized in Table 3.

The murine CMV was used as a model for studying the mechanism of this antiviral activity. Virus which had been treated with any of these compounds + UVA was still capable of penetrating susceptible mouse cells and gained entry to the cell nucleus, the normal site of replication of this virus. However, there was no evidence for viral gene expression, i.e. no viral DNA, RNA, or protein synthesis (Hudson et al., 1988a). Visnagin and 8-MOP were capable of cross-linking viral DNA, whereas angelicin and khellin were not (Altamirano et al., 1986). Thus it appears that the phototoxicity attributed to these compounds is due to photoadduct formation with the viral DNA, so that template activity is curtailed. Their variable degrees of antiviral activity may then reflect their relative efficacies in forming adducts with the DNA.

However, other targets and mechanisms cannot be ruled out, and in fact several recent reports have described photoactive effects of psoralens against proteins, including enzymes such as DNA polymerase (Granger et al., 1982), cell surface receptors (Laskin et al., 1985, 1986), and membrane fatty acids (Specht et al., 1988). Since many animal viruses contain these potential target molecules, it would be interesting to determine if furyl compounds could photodamage them.

β-Carboline alkaloids

More than 5000 alkaloids have been isolated, and chemically characterized, from plant sources. They are widely distributed, and many of them have biological activities, although few have been evaluated for antiviral or antimicrobial activities (Hudson, 1989). Some of them are also photosensitizers, a fact which was not appreciated during many of the early studies on bioactivities.

The harmane-related alkaloids (Fig. 6) have been found in 26 families of plants, as well as in animal cells (Melchior and Collins, 1982). They are synthesized in the plants from tryptophan.

Some of the β-carbolines have been shown to bind readily to nucleic acids (Smythies and Autun, 1969; Duportail and Lami, 1975; Hayashi et al., 1977) but the role of light was not considered in these studies. This is important, however, since these compounds do absorb significantly in the UVA region, and in fact recent studies have shown that some of them possess antimicrobial activities, in UVA (McKenna and Towers, 1981), and they can cause UVA-mediated chromosomal damage in cultured hamster cells (Towers and Abramowski, 1983). It was observed however that, unlike the structurally similar furocoumarins (Fig. 5), harmine did not form cross-links with DNA in the presence or absence of UVA (Altamirano et al., 1986).

Antiviral properties of β-carbolines

Antiviral (virucidal) activity of β-carboline alkaloids was observed for both MCMV and Sindbis virus. Table 4 shows the relative minimum inhibitory concen-

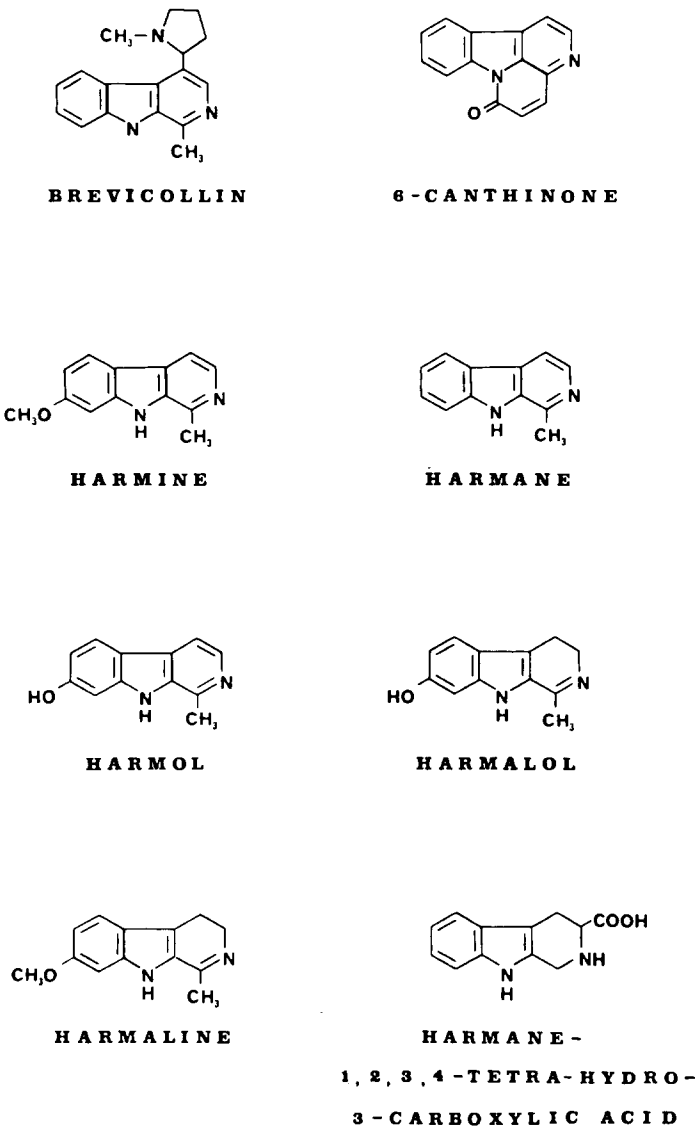


Fig. 6. Structural formulae of carboline alkaloids tested for antiviral activity.

trations (MIC) needed to inactivate 10^2 PFU of virus, (Hudson and Towers, 1988a).

The relative order of potencies against MCMV were harmine > harmol > (harmalol ≃ harmaline) > harmane > harmane-tetrahydrocarboxylate. The order of potencies against SV was slightly different, harmine > (harmalol ≃ harmaline) > harmol > harmane > tetrahydrocarboxylate. Under these stringent test conditions we were not able to detect antiviral activity for the related alkaloids brevicollin and 6-canthinone, which had previously been shown to possess phototoxic effects

TABLE 4

Relative antiviral activity of harmine-type alkaloids

Compound	MIC[a] (μg/ml) vs.	
	SV	MCMV
Harmine	0.23	1.87
Harmane	3.9	> 10
Harmol	1.17	3.44
Harmalol	0.29	7.50
Harmaline	0.35	8.44
Harmane tetrahydrocarboxylate	> 10	> 10
Brevicollin	> 10	> 10
6-canthinone	> 10	> 10

[a]MIC = minimum inhibitory concentration required to completely inactivate 10^2 PFU of virus.

in mammalian and bacterial cells (Towers and Abramowski, 1983). Perhaps these compounds could show some antiviral effect under less stringent conditions, i.e., less than 100% inactivation. Clearly they were not as effective as the harmane derivatives. Evidently minor changes in the structures of these alkaloids can have profound effects on their antiviral potency. Whether this is due to their different degrees or type of interaction with the viral nucleic acids, or to different abilities to gain access to the viral genome, is not known.

The MCMV was studied in more detail in order to elucidate the mechanism of the antiviral effect. Virus which had been inactivated by harmine + UVA was used to infect cultured mouse cells, and various stages in the virus replication cycle were examined. The inactivated virus readily penetrated into the nucleus of mouse cells; but no viral protein synthesis or RNA synthesis could be detected, and the viral DNA did not replicate. In contrast, virus which had been treated with harmine in the dark promoted a complete growth cycle in mouse cells (Hudson et al., 1986d,e).

An attempt was made to identify the primary target of harmine + UVA activity by examining the bacteriophages T4 and M13, which do not possess membranes. Both bacteriophages were sensitive but the single-stranded DNA phage M13 was considerably more so. These results strengthen the view that DNA and probably other macromolecules can serve as targets for harmine photoactivity, although the compound does not produce cross-links in DNA (Altamirano et al., 1986).

These observations, together with the fact that some harmane compounds have previously been shown to interact with DNA and RNA in light-independent reactions (Duportail and Lami, 1975; Hayashi et al., 1977), implies that the mechanism of photoinduced damage is different from that caused by the furanocoumarins. We must also entertain the possibility that alternative targets, such as specific virion proteins, also exist.

Recently a number of carboline derivatives was isolated from the Caribbean tunicate, *Eudistoma olivaceum* (Rinehart et al., 1987). We compared the antiviral phototoxicities of five of these eudistomins (Hudson et al., 1988b). Their activities were quite different, one being as potent as harmine while one was inactive. These differences were attributed to the effects of substituents (Hudson et al., 1988b).

In another recent study (Tanrisever and Towers, unpublished data), it was shown that harmine, in the presence of UVA, could interact with various polynucleotides, particularly well with single-stranded polypurine polymers, in a reaction which was independent of O_2. This preference for single-stranded polynucleotides could explain the greater susceptibility of Sindbis virus and phage M13 to the phototoxicity of harmine.

Other alkaloids

The furanoquinoline alkaloid dictamnine, which structurally resembles furocoumarins, (see Fig. 5), occurs in many species in the Rutaceae family of flowering plants, often in association with furocoumarins (Towers and Hudson, 1987). Several alkaloids of this type were shown to possess UVA-mediated phototoxicity toward bacteria, fungi and cultured cells (Pfyffer et al., 1982a; Towers and Abramowski, 1983). In further studies it was shown that dictamnine was able to bind to DNA, in the presence of UVA, in a reaction that resembled furocoumarins, such that template activity was abolished, although covalent adducts have not yet been described (Pfyffer et al., 1982b).

Dictamnine was found to be quite active (virucidal) against MCMV, almost as potent as 8-MOP; but less active against SV (Hudson et al., 1985). These activities required UVA. Virus retained its structural integrity however, and could penetrate mouse cells readily, although the viral DNA could not transcribe or replicate.

Thus the mechanism of the antiviral action appeared to resemble furocoumarins, rather than carbolines, suggesting that the furyl group, when present, can govern the mode of interaction with nucleic acids.

In a recent survey of various alkaloids for intracellular activities against different viruses, Vanden Berghe et al. (1986) found the following to have significant and broad-spectrum activity: codeine, cryptoleurine, emetine, lycorine, pretazettine, and scopolamine. Considering the relatively high concentrations of compounds used, however (maximum non-toxic doses), these activities were not really impressive, and in fact Vanden Berghe et al. (1986) tended to play down the antiviral results as consequences of non-specific inhibition of protein synthesis in the infected cells.

Some other alkaloids have been shown to possess antiviral activities (Becker, 1980) e.g. camptothecin, which possesses some structural similarity to carbolines and furocoumarins; atropine; and castanospermine; however, there does not appear to have been a study of their possible photoactivities (Hudson, 1989).

Conclusions

It is evident that many of the photosensitizers that belong to the groups of compounds studied by us have antiviral activity (summarized in Table 5). In all cases, their effects are strictly UVA-dependent. This might be thought of initially as a

TABLE 5

Summary of antiviral phototoxicity

Compound	Relative activity against			
	T4 ds DNA −membrane	M13 ss DNA −membrane	MCMV ds DNA +membrane	SV ss RNA +membrane
Furyl compounds				
8-methoxypsoralen	+++[a]	+++	+++	+
angelicin	++	+++	+	+
dictamnine	++	+	++	+
visnagin	++	+	++	+
khellin	+	+	+	±
β-Carbolines				
harmine	++	+++	++	+++
harmane derivatives and eudistomins			− to ++	− to +++
Thiophenes, polyacetylenes				
α-terthienyl	+	−	++++	++++
thiarubrin-A	+	−	+++	+++
thiophene-A	−		+	+
phenylheptatryine	−		++	++
other thiophenes			− to ++++	− to ++++

[a]Number of + indicates relative degree of phototoxicity; −, no detectable activity. (From Hudson and Towers, 1988.)

disadvantage, since therapy with these compounds would involve concomitant UVA irradiation. However, this is not necessarily a problem, in view of the fact that most tissues are accessible to focused laser beams, and in any case PUVA therapy (PSORALEN + UVA) has been successfully carried out for years by simply administering the compound to a patient who was then exposed to UVA lamps. The reasons for the gradual demise of PUVA was the mutagenic and carcinogenic potential of the psoralens.

The compound 8-MOP has also been used extracorporeally on separated leukocytes to control cutaneous T-cell lymphomas; the process being referred to as photopheresis (Edelson et al., 1987). In this procedure, blood is removed from the patient, the leukocytes are separated and passed through a long tube continuously irradiated by UVA (BLB) lamps. The treated cells are then returned to the patient. Similarly cancer chemotherapy with hematoporphyrin derivatives requires simultaneous light exposure. In fact, the UVA requirement may be considered a benefit since this ensures selectivity; in the absence of UVA these photosensitizers appear to be innocuous, and following the photoreaction they usually degrade to harmless products.

In view of these considerations, we believe that it is worthwhile examining some of these compounds in model animal virus infections. This will be the true test of

their practicality. It may also be feasible to conjugate these compounds to appropriate cell-specific molecules in order to target them to virus-infected tissues. This kind of approach was found to be successful in the control of an ascites tumor in mice following administration of hematoporphyrin-antibody conjugate and intermittent exposure to lamps (Mew et al., 1983). The light, in this case in the visible range, was essential.

The mechanisms of action, however, are clearly different among these groups described. Thus the furyl compounds probably exert action through the formation of photoadducts with the viral genome, which then results in a block of gene expression, although other viral macromolecules could be potential targets as well. In contrast the thiophenes and polyacetylenes probably act on virion membrane constituents, although again other targets cannot be discounted. The carbolines appear to be more complex and may act on several viral targets. In regard to therapeutic use, the thiophenes appear to be the most promising at present, because of their potency.

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