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Acridones As Antiviral Agents: Synthesis, Chemical and Biological Properties

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Abstract: Acridones are a class of compounds that have attracted attention in recent years for their wide range of biological properties, including selective inhibition of diverse human pathogenic viruses. The wide spectrum of antiviral activity includes DNA and RNA viruses, such as herpes simplex virus, cytomegalovirus, adenovirus, hepatitis C virus, dengue virus, and Junin virus, among others, indicative of the involvement of cellular factors as potential targets of acridone derivatives. At the present, their precise mode of action is not clearly determined, although the predominant action seems to be centered on the synthesis of nucleic acids. Regarding this point, inhibitory activity against cellular and viral enzymes and the ability to intercalate into nucleic acid molecules was demonstrated for some acridone compounds. Then, the possibility of a multiple effect on different targets renewed interest in these agents for virus chemotherapy allowing a potent inhibitory effectiveness associated to less feasibility of generating antiviral resistance. This review summarizes the current knowledge regarding the methods of synthesis, the antiviral properties of acridone derivatives, their mechanism of action, and structural characteristics related to antiviral activity as well as the perspectives of this class of compounds for clinical application against human viral infections.

Keywords: Acridones, antiviral activity, herpes simplex virus, flavivirus, arenavirus, cellular target.

1. INTRODUCTION

Acridones are a subclass of acridines with a basic structure consisting of 9(10*H*)-acridone (Fig. **1**) which is present in a large number of natural products and synthetic compounds that have been known as multitargeted agents with a wide spectrum of biomedical potential due to their diverse and atypical mechanisms of action. Acridone-based derivatives were first pursued for their antimicrobial properties against bacteria, parasites and fungi [1, 2]. Some work in this area continues, particularly for the development of new chemotypes active for treatment of the malaria agent *Plasmodium falciparum* or trypanocidal agents [3-7]. However, more recent research has focused mainly on their use as anticancer drugs, due to their planar structure that confers the ability to intercalate within DNA and therefore to interfere with metabolic processes in living cells [8-10]. Given their capability of binding to nucleic acids, the antiviral properties of acridones against DNA viruses and, more lately, RNA viruses also were analyzed and have attracted attention in recent years. The diverse and varied biological activities of such a heterocyclic ring have led several authors to write review articles [11-13]. In this work, we summarize the traditional methods of synthesis as well as the new synthetic advances, the spectrum of antiviral activity of acridones and chemical characteristics related to antiviral activity of selective virus inhibitors, the mechanism of action of active compounds as well as the perspectives of this class of heterocyclic compounds for clinical application against relevant human viral infections.

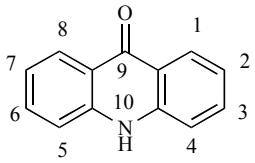

Fig. (1). Acridone basic structure and numbering system.

2. SYNTHESIS AND CHEMICAL PROPERTIES

The acridone molecule is planar with no atoms deviating by more than 0.02 Å from the molecular plane defined by non-H ring atoms and the oxygen atoms. All torsion angle lies within +1.5 to -1.5 of 0 to 180 degree. The molecular packing arrangement in acridones is characterized by two major interaction types: (a) N-H…O hydrogen bonds between glide-related molecules, with an N…O distance of 2.782 Å, such that each molecule is hydrogen-bonded to two adjacent molecules; and (b) interaction between molecules stacked along a short crystal axis [14].

Several acridone alkaloids have been isolated from diverse plant genera. Particularly, the acridone derivatives of the genus *Citrus* [15-19] present a similar substitution pattern. The main difference lies in the presence of an isoprene group (cyclic or not) instead of hydrogen or methoxy group

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in the C-4 of the acridonic ring. The structures of the acridone alkaloids from *Citrus* plants are presented in (Fig. **2**), which includes a potent antiherpetic compound (**6**) known as citrusinine-I.

Similar structures were isolated by Rastogi *et al.* [20] from *Glycosmis mauritiana*, where the isoprene group was located either in position 4 or in position 2 of the tricycle (compounds **15** and **16**) (Fig. **3**). Chansriniyom *et al.* [21] isolated six acridone alkaloids from the branches and the leaves of *Glycosmis parva* CRAIB collected in the east of Thailand, two of them (compounds **19** and **20**) showed antiviral activity against herpes simplex virus (HSV) types 1 and 2. Interesting compounds, such as *N*-methyl-severifoline (**21**), severifoline (**22**), atalaphyllinine (**23**), *N*-methylata laphylline (**17**) and acridone **24** were isolated from *Severina buxifolia* [22]. These three last alkaloids were also obtained from the *Atalantia monophylla correa* [23]. Furthermore, treatment of compound **21** with 85% formic acid at 80-90 °C for 3 h gave compound **26**. Compound **25** was obtained by methylation of **21**.

Due to the different and important biological activities of this kind of heterocycles, a great number of research groups have worked on the development of efficient syntheses. The traditional methodology to obtain the acridone ring is the Ullmann synthesis involving the condensation of *o*-halobenzoic acids with substituted anilines to give *N*-(substituted phenyl)anthranilic acids. The cyclization of these last ones under strong acid conditions produces the acridone ring (Fig. **4**).

The Ullmann reaction is catalyzed by a variety of forms of copper, including the metal itself (in various forms: copper, bronze, freshly precipitated, "activated", etc), salts or complexes of cuprous or cupric ion, and insoluble oxides. Reaction conditions such as: a) potassium acetate, cuprous acetate in water, triethylamine, 2-propanol at reflux; b) copper, potassium carbonate in dimethylformamide at 60–120 °C, during two hours; or c) cuprous iodide, pyridine, potassium carbonate, water at reflux have been reported [24]. Additionally, unconventional energy sources, as for example ultrasound [25] or microwave (MW) [26, 27] irradiation, have also been reported. These methods may reduce both the energy consumption and essentially reaction times.

The thermal cyclization of anthranilic acids involves harsh reaction conditions, such as refluxing with polyphosphoric acid, phosphorous oxychloride in acetonitrile or sulfuric acid. However, recently Pal *et al.* [28] described the use of Eaton's reagent (a mixture of phosphorus pentoxide and methansulfonic acid) to promote this cyclization under more soft reaction conditions.

Most acridonic derivatives which showed important antiviral activity were synthesized from anthranilic acid obtained by the Ullmann reaction. The biologically active acridone-4-carboxamides were synthesized from acridone-4-carboxyl chloride with alkyl, aryl or heteryl amines [29]. This carboxyl chloride compound was obtained by treatment of the corresponding carboxylic acid synthesized using a Jourdan–Uilman copper-catalysed reaction with thionyl chloride. More recently, the same group described the synthesis of (2-halo)-5-methoxyacridone-4-carboxylic acid, under Uilman conditions, and the carboxamides resulting from the treatment of this acridonic acid with 2-, 3- or 4-aminopyridines or 3-, 4-, 5-trimethoxyaniline [30], for example compound **29** (Fig. **5**).

A series of acridones with various substitution patterns, like a hydroxyl or methoxy group at the C-1 and/or C-3 position, a methyl group at the *N*-10 position, in the C-7 position an amino group or its nitro precursor, and the C-6 position with a 4-(2-pyridinyl)-1-piperazinyl side chain, were synthesized from the 7-nitro-6-chloro-1,3-dimethoxy-10-methyl-9(10*H*)-acridone **30** (Fig. **6**) [31]. This last compound was obtained by Ullmann reaction of 2,4-dichloro-5-nitrobenzoic acid with 3,5-dimethoxyaniline [32].

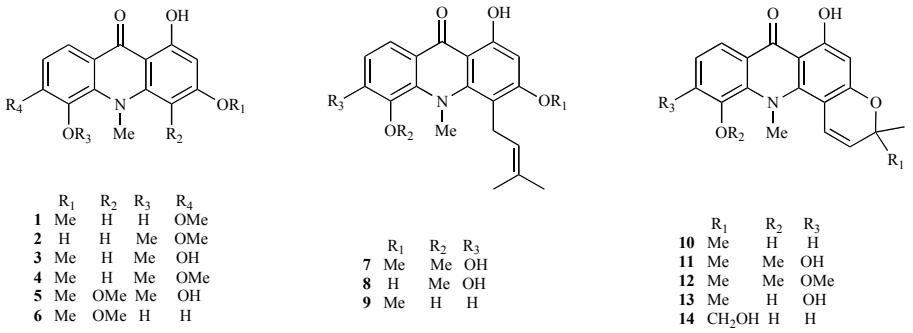
The 7-amino-9-acridone derivatives were obtained by following four sequential steps: *N*-alkylation, nucleophilic substitution at C-6 with selected nucleophiles, reduction of nitro group, and finally de-*O*-methylation. The nucleophilic reaction of acridone **30** with 1-(2-pyridinyl)-piperazine afforded the corresponding 6-[4-(2-pyridinyl)-1-piperazinyl] derivative **31a-c** shown in (Fig. **6**). The reduction step was performed using tin chloride under acidic conditions from nitro derivatives. The 1,3-dihydroxy and 1-hydroxy-3-methoxy series were synthesized by treatment of the corresponding 1,3-dimethoxy derivatives with 48% bromhydric acid in the presence of traces of acetic acid and the progress of this reaction was monitored to avoid the formation of the sole dihydroxy compound.

The same synthetic scheme was applied in order to obtain *N*-methyl acridones like **31** but substituted in C-6 with different 4-arylpiperazines [33]. Also, a methoxy group in the C-6 position may be obtained using sodium methoxide as the nucleophile. Other 1-alkoxy derivatives were prepared by *O*-alkylation with the selected alkyl halide from the 1-hydroxy-3-methoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridone.

The synthesis of symmetrical bisimidazoacridones like **32** (Fig. **7**) was described by Cholody *et al.* [34]. The starting materials were the 1-chloro-4-nitroacridone derivatives obtained from known 2-(phenylamino)-6-chloro-3-nitrobenzoic acids according to previously reported methods [35, 36]. The acridones were condensed with N^2-methyldiethylenetriamine or 3,3'-diamino-*N*-methyldipropylamine in dimethyl sulfoxide in the presence of triethylamine to give bisnitroacridones which were transformed into bisimidazoacridones by refluxing in formic acid in presence of aluminum-nickel alloy. On the other hand, the mono-substituted compound obtained in reaction of l-chloro-4-nitroacridone with an excess of 3,3'-diamino methyldipropylamine was condensed with a suitable acridone to give asymmetrical bisnitroacridones or was transformed into mono-imidazoacridone by refluxing in formic acid in presence of aluminum-nickel alloy.

Likewise, condensation of 5-chlorotriazoloacridone with 1,4-bis(3-aminopropyl)piperazine in dimethyl sulfoxide (DMSO) at 100 °C yields the 1,4-bis[3-(6-oxo-6H-v-triazolo [4,5,1-de]acridin-5-yl)amino-propyl]piperazine (**33**), known as temacrazine [37].

The Ullmann reaction between a 2-chlorobenzoic acid and a 3-substituted amine is not usually used because it

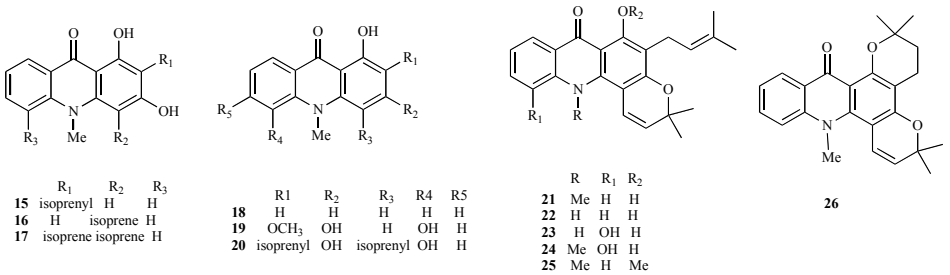


	R_1	R_2	R_3	R_4
1	Me	H	H	OMe
2	H	H	Me	OMe
3	Me	H	Me	OH
4	Me	H	Me	OMe
5	Me	OMe	Me	OH
6	Me	OMe	H	H

	R_1	R_2	R_3
7	Me	Me	OH
8	H	Me	OH
9	Me	H	H

	R_1	R_2	R_3
10	Me	H	H
11	Me	Me	OH
12	Me	Me	OMe
13	Me	H	OH
14	CH_2OH	H	H

Fig. (2). Acridone derivatives isolated from plants of the genus *Citrus.*



	R_1	R_2	R_3
15	isoprenyl	H	H
16	H	isoprene	H
17	isoprene	isoprene	H

	R1	R_2	R_3	R4	R5
18	H	H	H	H	H
19	OCH_3	OH	H	OH	H
20	isoprenyl	OH	isoprenyl	OH	H

	R	R_1	R_2
21	Me	H	H
22	H	H	H
23	H	OH	H
24	Me	OH	H
25	Me	H	Me

26

Fig. (3). Acridones isolated from *Glycosmis mauritiana, Glyscosmis parva* and *Atalantia monophylla correa*.

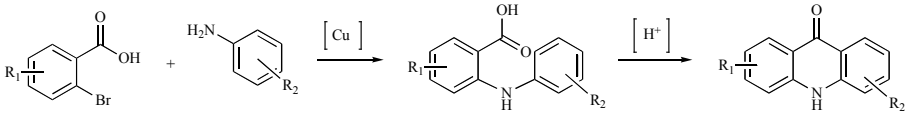
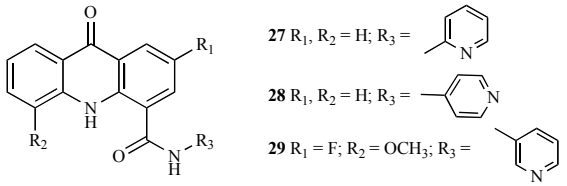


Fig. (4). General synthetic pathway to obtain acridone ring using Ullmann reaction.

yields a mixture of 1- and 3-substituted products, which cannot be separated easily.



27 R_1, R_2 = H; R_3 =

28 R_1, R_2 = H; R_3 =

29 R_1 = F; R_2 = OCH_3; R_3 =

Fig. (5). Acridone-4-carboxamide structures.

Goodell *et al.* [38] described the synthesis of acridone-3-carboxilic acid starting from aniline and 2-bromoterftalic acid and using phosphorus oxychloride to generate the unstable 9-chloroacridine intermediate which was then hydrolyzed in hydrochloric acid (1M) yielding the carboxyacridone.

N-substituted acridones were prepared by treatment to the corresponding acridone in basic medium (sodium hydride) with an alkyl halide in presence of phase transfer agent, such as tetrabutylamonium iodide. As shown in (Fig. **8**), this procedure was applied to obtain compound **35** from **34** by treatment with bromocyclopropane [39].

Series of *N*-allyl, *N*-allenyl and *N*-(3-methyl-2-butenyl)-9(10*H*)-acridones **36-38** were synthesized using the same methodology [40-42]. Isoxazoline derivatives **39** resulting from the 1,3-dipolar cycloaddition between *N*-allyl derivatives **36** and the sugar oximes in presence of chloramine-T were also obtained (Fig. **9**).

Similarly, ethyl 2-(9-oxo-9,10-dihydro-10-acridyl)acetate was obtained using ethyl 2-bromoacetate as *N*-alkylating agent. The saponification of ethyl ester and subsequent acidification produces 10-carboxymethyl-9-acridone (**40**, CMA).

Ning *et al.* [36] reported that CMA reacted with thionyl chloride giving an unusual intermediate **41** (Fig. **10**), whose structure was established by x-ray crystallography. Intermediate **41** is very readily cleaved by protonic reagents. Thus, on reaction with ethylene glycol and dodecanol, followed by brief exposure of the reaction mixture to water during isolation of the products, the chlorosulfinyl group of **41** was lost and formed esters of the acridanacetic acids. Under mild methanolysis conditions, the authors isolated an unstable methyl 9-oxo-α-sulfinyl-10-acridanacetate. This last compound was capable of losing the sulfinyl group by mild hydrolysis to afford methyl 9-oxo-10-acridanacetate.

Acridones fused with a heterocyclic ring have been synthetized by Ullmann condensation. Taraporewala [43] obtained thiazoloacridones from benzotriazole derivatives.

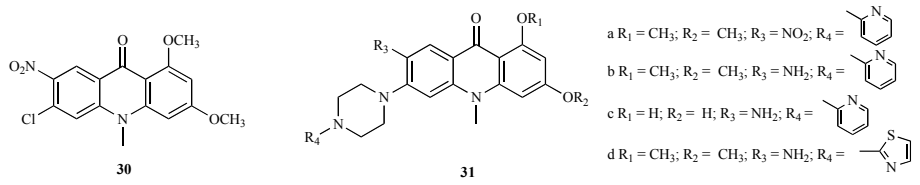


Fig. (6). Acridone derivatives 1,3 substituted with oxygenated groups.

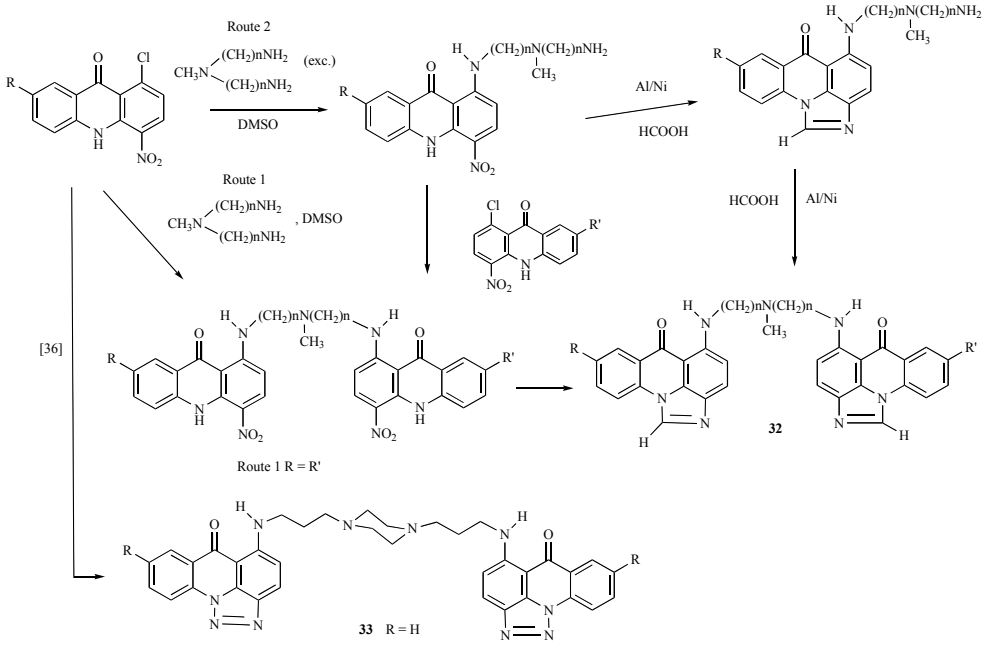


Fig. (7). Synthetic scheme to obtain mono- and bisimidazoacridones.

Others have been synthesized using an acridone derivative as starting material. Analogues of dercitin, an antitumor and antiviral marine pyrido[4,3,2-mn]thiazolo[5,4-b]acridine, was prepared by treatment of 2,3-diaminoacridone with excess cyanogens bromide (Fig. **11**) [39].

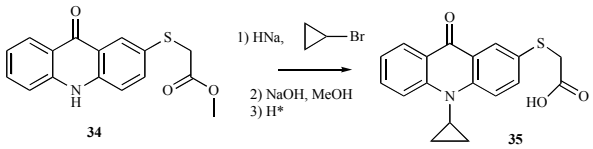


Fig. (8). Synthesis of *N*-cyclopropyl acridone derivative.

The Ullmann reaction is not effective for synthesis of the 1,3-dihydroxyacridone derivatives, which displayed important antiviral properties. In this case, the synthesis was performed by heat to reflux an equimolar mixture of 2-aminobenzoic acid derivative and anhydrous phloroglucinol in the presence of anhydrous zinc chloride in *n*-butyl alcohol (Fig. **12**).

This reaction is believed to go through a Friedel-Crafts-like mechanism for the initial electrophilic aromatic substitution and subsequently forms the acridone system through hydrolytic cyclization between the primary amine and the hydroxyl adjacent to the substitution [35]. Under these conditions, the reaction gave generally low yields or failed when 3-substituted anthranilic acids were used. Best yields were obtained when the coupling was performed heating an equimolar mixture of the starting material to 230 °C for 35 min. The main advantage was that the products could be obtained in one step with a simple purification [44]. This methodology was extended to other resorcinol derivatives, as 5-methoxyresorcinol, and the resorcinol itself. Starting from the appropriate anthranilic acid, a series of synthetic 1-hydroxyacridones presented in (Fig. **13**) could be obtained [45].

The majority of synthetic approaches are regioselectivity-compromised, and usually require harsh, acid mediated conditions. In (Fig. **14**) is shown an attractive acridone synthetic method that consists of the treatment of *N*-methylated diarylamines with lithium diisopropyl amide (LDA) at 0 °C to produce acridones in moderate to excellent yields. Pd-mediated C-N bond formation coupling methods had been employed to obtain the *N*-methylated diarylamines precursors [46].

In recent works, two new acridone syntheses were reported using, in both cases, aryne precursors (Fig. **15**). First, was described the reaction of silylaryl triflates in the presence of fluorure donant with methyl 2-(*N*-methylamino) benzoate, which produces an acridone ring in an efficient one-step synthesis [47]. In a second work, Fang *et al.* [48] described the reaction between β-lactams with a molecule of

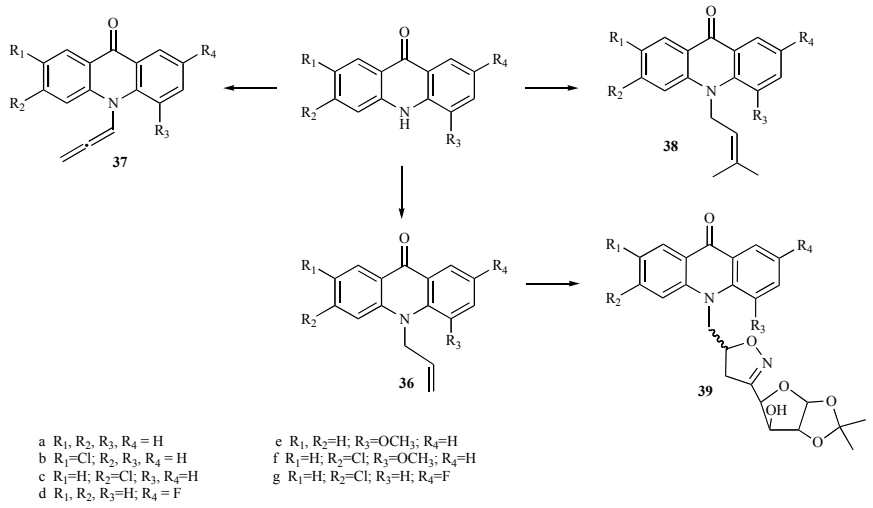


Fig. (9). Acridone derivatives obtained from *N*-alkylation with unsaturated groups and their posterior derivatization.

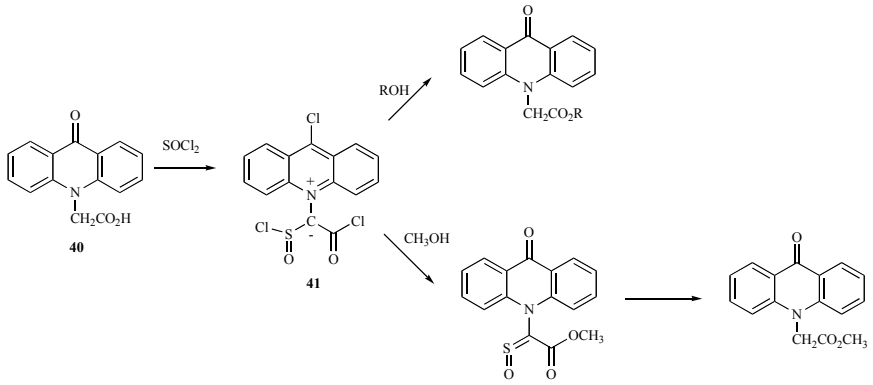


Fig. (10). Synthetic pathway to obtain CMA derivatives.

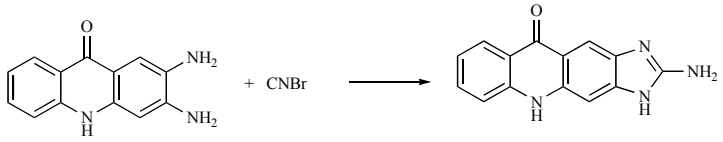


Fig. (11). Synthesis of tetracylic derivatives.

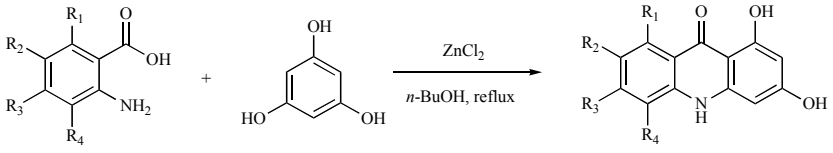
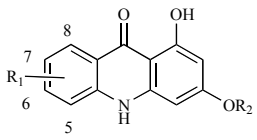


Fig. (12). Synthetic scheme to obtain 1,3-dihydroacridones.

aryne by insertion into the amide bond to form a 2,3-dihydroquinolin-4-one, which subsequently reacts with another molecule of aryne to form an acridone by extrusion of a molecule of ethylene. Simultaneously, the same insertion of arynes into the C-N bond of *N*-unsubstituted-β-lactams was described [49].

3. SPECTRUM OF ANTIVIRAL ACTIVITY

Concerning the antiviral properties of this class of compounds, several studies have shown the inhibitory action of acridone derivatives of natural and synthetic origin against diverse viruses with double-stranded DNA genomes as well as RNA viruses. The degree of inhibitory activity varies with



42 R_1 = 7-OMe, R_2 = Me
43 R_1 = 7-OMe, R_2 = H
44 R_1 = 7-OMe, R_2 = allyl
45 R_1 = 7-OH, R_2 = Me
46 R_1 = 5-OMe, R_2 = Me
47 R_1 = 5-OMe, R_2 = H
48 R_1 = 7-OH, R_2 = H
49 R_1 = H, R_2 = H
50 R_1 = 5-Cl , R_2 = H
51 R_1 = 6-Cl , R_2 = H
52 R_1 = 7-Cl , R_2 = H
53 R_1 = 8-Cl , R_2 = H
54 R_1 = 5,7-diCl , R_2 = H
55 R_1 = 5-Cl , R_2 = Me
56 R_1 = 5-Me , R_2 = H
57 R_1 = 8-Me , R_2 = H

Fig. (13). Several 1-hydroxyacridone analogues prepared by condensation of anthranilic acid and resorcinol derivatives.

the compound and the virus. The spectrum of antiviral activity of a diverse set of acridone derivatives are shown in (Table **1**), representing the most active compounds for each structural group.

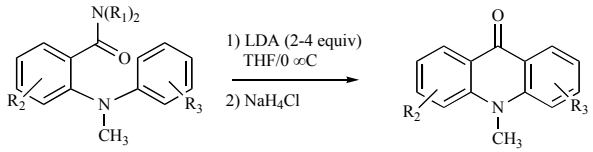


Fig. (14). Basic cyclization conditions.

3.1. DNA Viruses

The initial and also most extensive studies regarding antiviral activity of both natural and synthetic acridones against DNA containing viruses were performed with herpes simplex virus (HSV). There are two HSV serotypes (HSV-1 and HSV-2) that may cause oral disease, keratoconjuntivitis, encephalitis, and genital infections [50]. Most compounds in clinical use are nucleoside analogues, with acyclovir as an excellent therapeutic drug. Acyclovir acts through the function of two HSV enzymes: first, the viral thymidine kinase, which selectively phosphorylates acyclovir to the monophosphate form, then the diphosphate and triphosphate are subsequently formed by the action of cellular kinases. The second enzyme is the viral DNA polymerase, which is the target inhibited by acyclovir triphosphate by a mechanism known as obligate chain termination [51]. This highly selective mechanism of action is the basis of the therapeutic success of acyclovir for HSV treatment. However, acyclovir-resistant strains frequently emerge during long term antiviral treatment in immunocompromised patients [52], and therefore the discovery of new non-nucleoside antiviral compounds is of significant interest [53, 54].

The majority of research regarding the antiviral properties of acridones against herpesviruses was performed with different series of natural and synthetic derivatives of 1-hydroxiacridones. A pioneer study identified several alkaloids isolated from the root bark of the plant species of the genus *Citrus* (Rutaceae) as inhibitors of both HSV-1 and HSV-2 [55]. Citrusinine-I (**6**), a compound belonging to the 1-hydroxyacridone subclass, was the most potent alkaloid with effective concentration 50% (EC_{50}), the concentration required to inhibit virus plaque formation by 50%, of 0.56 and 0.74 μg/ml against HSV-1 and HSV-2, respectively. This compound was also active against human cytomegalovirus (HCMV), another herpesvirus pathogen of humans, with an EC_{50} of 1.5 μg/ml. Although this spectrum of activity against the three herpesviruses more prevalent in human infections is a remarkable property of citrusinine-1, not usual for the majority of approved anti-herpetic drugs, the selectivity of this compound was moderate. The selectivity index (SI) is defined as the relationship between cytotoxicity, measured as the cytotoxic concentration 50% (CC_{50}: drug concentration required to reduce cell viability by 50%), and the antiviral EC_{50}. As seen in (Table **1**), the values of SI for these phytochemicals did not reach a relationship of 20-fold. In another study, three acridone alkaloids isolated from branches and leaves of *Glycosmis parva* (Rutaceae), denominated glycosparvarina, glycofolinina (**5**) and *S*-deoxydihydroglyparvin, showed anti-HSV-1 activity but also at concentrations very close to the cytotoxic doses [21]. Interestingly, a different series of acridone alkaloids isolated from diverse Rutaceous plants exhibited a marked inhibitory effect on activation of Epstein-Barr virus, another human herpesvirus, leading to tumor-promoting activity, with promising perspectives for some prenylated acridones as cancer chemopreventive agents [56, 57].

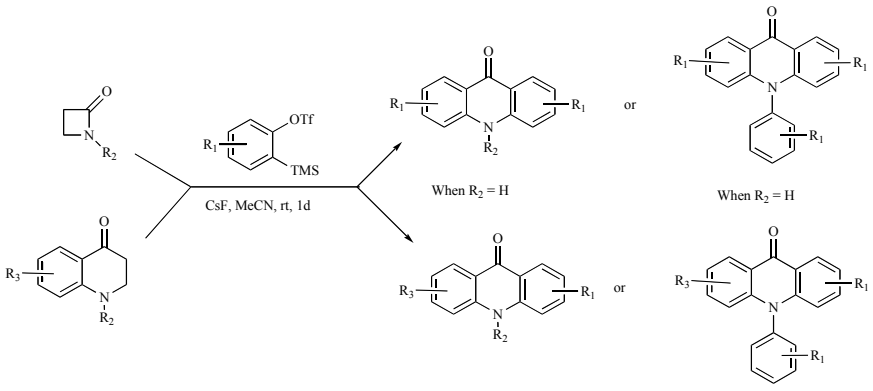


Fig. (15). Synthetic scheme of acridones from β-lactams or 2,3-dihydroquinolin-4-ones with arynes.

Table 1. Spectrum of Antiviral Activity of Natural and Synthetic Acridones

Compound	Figure	SI[a]	Virus	Reference
Acridone alkaloids				
Citrusinine-I (**6**)	2	>7->18	HSV-1, HSV-2, HCMV	[55]
Glycofolinine (**5**)	2	2-3	HSV-1, HSV-2	[21]
1-Hydroxyacridones				
5-Chloro-1,3-dihydroxyacridone (**50**)	13	13-26	HSV-1	[58]
7-Chloro-1,3-dihydroxyacridone (**52**)	13	4-8	HSV-1	[58]
1,3-Dihydroxy-5-methoxyacridone (**47**)	13	13-50	HSV-1, HSV-2	[44]
7-Amino-1,3-dihydroxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*) acridone (**31c**)	6	>200	BVDV	[31]
3,7-Dimethoxy-1-hydroxyacridones (**42**)	13	>36	HCMV	[45]
3-Allyloxy-7-methoxy-1-hydroxyacridone (**44**)	13	>20-41	HSV-1, HSV-2, HCMV	[45]
1-Hydroxy-10-methyl-9(10*H*)-acridone (**18**)	3	9	HIV-1	[62]
10-Allyl-9(10*H*)-acridones				
10-Allyl-6-chloro-4-methoxy-9(10*H*)-acridone (**36f**)	9	>213->323	DENV, JUNV	[42]
10-Allyl-6-chloro-9(10*H*)-acridone (**36c**)	9	>181->400	DENV, JUNV	[42]
Acridone-4-carboxamides				
N-(Pyridin-2-yl)-acridone-4-carboxamide (**27**)	5	40	HCV	[29]
N-(Pyridin-4-yl)-acridone-4-carboxamide (**28**)	5	19	HCV	[29]
2-Fluoro-5-methoxy-*N*-(pyridine-3-yl)-acridone-4-carboxamide (**29**)	5	>28->1000	HCV	[30]
Other acridones				
7-Amino-1,3-dimethoxy-10-methyl-6-[4-(4-thiazol)-1-piperazinyl]-9(10*H*) acridone (**31d**)	6	>17	HCV	[33]
Temacrazine (**33**)	7	>21->2000	HIV-1	[37]
N-Cyclopropyl-acridone-2-thioacetic acid (**35**)	8	32	HIV-1	[39]

[a]SI (selectivity index): ratio between cytotoxic concentration 50 % (CC_{50}), concentration required to reduce cell viability by 50%, and effective concentration 50% (EC_{50}), concentration required to inhibit virus activity by 50%.

In an attempt to solve the serious problem of selectivity failure presented by these natural acridone alkaloids, research focused on the development of synthetic 1-hydroxyacridones that may have simpler structure and better selective antiviral profile than the plant-extracted products. Several series of this acridone subclass were synthesized and characterized for their anti-herpetic activities, structure-activity relationships and inhibitory mechanisms. The first group included 1,3-dihydroxyacridone derivatives and two compounds, the 7-chloro-1,3-dihydroxyacridone (**52**) and the 5-chloro-1,3-dihydroxyacridone (**50**), appeared as promising lead anti-HSV agents [58]. Both acridones inhibited productive HSV-1 infection with a level of variable selectivity according to the experimental conditions: a more specific HSV inhibition with lower cell toxicity was obtained in rapidly dividing host cells than in resting cultures, increasing SI by 2-fold up to a maximum value of 26. Surprisingly, there was no a primary effect on HSV DNA replication since infection was blocked at a late step of the viral cycle [59]. The 1,3-dihydroxyacridone derivatives were also tested against HCMV replication but none of them were active [58]. Studies were then extended to a new set of 1,3-dihydroxyacridone analogues through modification of key functional groups in the acridone skeleton. The assay of these derivatives against HSV-1 and HSV-2 allowed the identification of 1,3-dihydroxy-5-methoxyacridone **(47)** as a significantly improved inhibitor with SI values of 13 and 50 for HSV-1 and HSV-2, respectively [44]. A third series of the 1-hydroxyacridone subclass was finally synthesized leading to variable 3,7-dialkoxylated derivatives with interesting properties. Among these compounds, the 3-allyloxy-7-methoxy-1-hydroxyacridone (**44)** appeared as the prototype of a new broader spectrum anti-herpetic agent which inhibited replication of HSV-1, HSV-2 and HCMV with comparable levels of selectivity [45]. In spite of these attractive properties, no

further improvement of 1-hydroxyacridones as candidates for HSV therapy was reported.

Another study reported the synthesis and activity of a group of acridones containing larger steric substitutions in the 3-position. Although the results with respect to antiviral activity of these compounds were interesting since it was the first report of acridones that were inhibitors of HSV-1 and inactive against HSV-2, the SI values for HSV-1 were even lower than those of the aforementioned 1-hydroxy-derivatives [38].

Cycloferon (10-carboxymethyl-9-acridanone, CMA) is a synthetic derivative of acridone close in chemical structure to some alkaloids from *Citrus* plants and well-known as a low molecular weight inducer of interferon [60]. However, CMA may also act by another mechanism of action through a direct antiviral effect independent of interferon. As such, a moderate antiviral effect of CMA against human adenovirus (HADV) infection was reported, but this preliminary study was not further pursued [61].

3.2. RNA Viruses

Among viruses with an RNA genome, a few studies have reported that the replication of human immunodeficiency virus type 1 (HIV-1), the lentivirus responsible for acquired immunedeficiency syndrome global epidemics, was inhibited at the transcriptional level by acridone derivatives. The 10-methyl-1-hydroxy-9(10*H*)-acridone (**18**) suppressed HIV-1 expression in latently infected cells as well as in acutely infected peripheral blood mononuclear cells with SI values around 9 [62]. A bistriazoloacridone analogue named temacrazine was found to inhibit very selectively virus replication in cells chronically and latently infected with HIV-1: antiviral EC_{50} values were in the range 0.1-10 nM while cytotoxicity was observed at concentrations approximately 1,000-fold higher, in the range of 1 to 10 μM [37]. Temacrazine **(33)** also inhibited acute infections with all strains of HIV-1 tested, including strains resistant to reverse transcriptase inhibitors in clinical use like zidovudine, nevirapine and didanosine. However, temacrazine failed to inhibit replication of HIV-2 or simian immunodeficiency lentiviruses, demonstrating that the antiviral action of this drug is highly specific for HIV-1 in the family *Retroviridae*.

In recent years, a very effective and selective action of acridones was detected with several pathogenic viruses belonging to the families *Flaviviridae* and *Arenaviridae*. Three members of *Flaviviridae* were successfully inhibited by diverse acridone derivatives. The first evidence of antiviral susceptibility was obtained with bovine viral diarrhea virus (BVDV), the prototype of the genus *Pestivirus* in *Flaviviridae* and a severe animal pathogen causing major losses in cattle throughout the world [63]. Several compounds of a small series of 10 acridone derivatives structurally characterized by hydroxyl or methoxy group at C-1/C-3 positions, methyl group at *N*-10 and amino or nitro group at C-7 elicited selective anti-BVDV activity determined by inhibition of viral cytopathogenicity in the bovine cell line MDBK [31]. The most potent inhibitor, 7-amino-1,3-dihydroxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridone (**31c**), was able not only to protect cells from virus-induced cytopathic effect with a SI higher than 200 but also reduced the production of infectious virus and extracellular viral RNA. Consequently, the authors postulated that viral RNA synthesis may be the antiviral target. In a later paper, these compounds and an enlarged series of new derivatives obtained by modifying the substitution pattern on the acridone nucleus was evaluated against hepatitis C virus (HCV), another member of *Flaviviridae* and agent of human chronic hepatitis affecting about 180 million people worldwide at high risk to develop liver cirrhosis and hepatocellular carcinoma [64]. The evaluation of the ability to inhibit HCV replication using human hepatic Huh-5-2 cells, a Huh-7 derived cell line containing an HCV subgenomic replicon with the luciferase reporter gene, led to the identification of new virus inhibitors [32]. However, given the relatively low SI exhibited by these chemotypes (Table **1**), their potency and selectivity must be increased to be developed as anti-HCV agents. Interestingly, a thiazolpiperazinyl derivative (**31d**) with antiviral activity determined by the replicon system was also an inhibitor of the HCV NS3 helicase activity, a very promising target for development of anti-HCV drugs since it is an essential enzyme in the virus replicative cycle and bears little homology to cellular helicases [65].

Another set of acridone 4-carboxylic acid derivatives, closely related to the above mentioned acridones, was also tested for inhibitory activity against HCV through different experimental approaches (effect on helicase activity of HCV, the transcription based on DNA-dependent T7 RNA polymerase, their intercalatory properties into double-stranded DNA and RNA, cytotoxicity and inhibition of RNA amplification of HCV replicon in Huh-7 cells). Two compounds, *N*-(pyridin-4-yl)-amide (**28**) and *N*-(pyridin-2-yl)-amide (**27**) of acridone-4-carboxylic acid, were efficient HCV RNA replication inhibitors in the subgenomic HCV replicon system expressed in hepatoma Huh-7 cells with a selectivity index of 19.4 and 40.5, respectively [29]. Both carboxamides were shown to be good helicase inhibitors and moderate DNA-dependent T7 RNA polymerase transcription inhibitors. Therefore the authors postulated that their inhibition of HCV replication in the replicon system could be due to a double effect of inhibition of two non-structural (NS) HCV enzymes, the NS3 helicase and the NS5B polymerase. In order to improve the inhibitory performance, the same group of researchers synthesized a new series of derivatives of 5-methoxyacridone-4-carboxylic acid. Among several active compounds, the 2-fluoro-5-methoxy-*N*-(pyridine-3-yl)-acridone-4-carboxamide **(29)** interfered with NS3 helicase activity and was the strongest inhibitor of HCV replication in a dose dependent manner and with very low cytotoxicity: the antiviral effective concentration 50% was 0.98 μM and the SI >1000, suggesting it is a key lead compound for further rational design of improved HCV inhibitors [30]. Interestingly, the combination of this acridone with either interferon or ribavirin, the only agents in clinical use to treat HCV patients [66], was additive, indicating the lack of interference in drug metabolism and a different mode of action and highlighting the good prognostic for acridones as anti-HCV therapeutic agents.

Dengue virus (DENV), the third flavivirus reported susceptible to acridone inhibition, is a human pathogen transmitted to its vertebrate host through the bite of an infected mosquito from the genus *Aedes*. At present, DENV is the

most widespread arbovirus in the world; with an estimated occurrence of 50 to 100 million human infections per year that can be manifested by a benign febrile illness called dengue fever or by a severe and potentially fatal disease named dengue hemorrhagic fever [67]. A screening of antiviral activity of diverse novel *N*-substituted acridone derivatives identified a group of 10-allyl-9(10*H*)-acridones as effective and very selective inhibitors of DENV [42]. In particular, the derivatives 10-allyl-6-chloro-9(10*H*)-acridone (**36c**) and 10-allyl-6-chloro-4-methoxy-9(10*H*)-acridone (**36f**) were the most potent compounds that blocked replication of the four serotypes of DENV in monkey Vero cells, with high selectivity (Table **1**). Noticeably, another hemorrhagic fever-causing RNA virus, Junin virus (JUNV), was also screened with this set of compounds and found almost equally susceptible to acridones as DENV [42]. JUNV belongs to the family *Arenaviridae* and is the causative agent of Argentine hemorrhagic fever, with mortality rates ranging from 10 to 20% in the absence of the administration of standardized doses of convalescent plasma that is today the only available therapy [68]. Acridones were effective inhibitors not only of JUNV but also of other pathogenic arenaviruses like lymphocytic choriomeningitis virus (LCMV). Furthermore, both acridones failed to inactivate virions before cell infection as well as to induce a refractory state by cell pretreatment, indicating that inhibition was exerted through a blockade in virus multiplication during the infectious process [42].

4. MECHANISM OF ACTION

The precise mechanisms of action of antiviral acridones are not clearly determined at present. Although the predominant mode of action seems to be centered on their ability of nucleic acid intercalation and, consequently, disruption of enzyme recognition and /or association to the modified nucleic acid [69], several cellular and viral enzymes have been also reported as direct targets for these compounds (Table **2**).

Table 2. Mechanism of Action of Acridones

Potential Targets	Virus	Reference
Cellular targets		
DNA topoisomerase type II	HSV	[38, 44, 58]
DNA cleavage and packaging	HSV	[59]
DNA replication and packaging	HADV	[73]
Transcription through PKC inhibition	HIV-1	[62, 74]
IMPDH	JUNV	[77]
Viral targets		
Viral ribonucleotide reductase	HSV	[55]
Integrase	HIV-1	[37]
RNA helicase / RNA polymerase	HCV, BVDV	[29-31]
RNA intercalation	HCV	[29, 30]

4.1. Cellular Targets

The type II DNA topoisomerases are essential nuclear enzymes that resolve topological problems in cellular DNA as they occur during replication, transcription, chromosome mechanics and recombination. This enzyme (the p170 isoform) localizes to HSV replication compartments at early times post-infection and biochemical analysis showed that it is preferentially engaged and localized non-randomly on progeny viral DNA during the later stages of infection [70, 71]. Although the function of DNA topoisomerase II in HSV-infected cells is not yet understood, this enzyme has been extensively studied as a candidate pharmacological target for treating herpesvirus infections [72].

In this context, the synthetic acridone derivative 7-chloro-1,3-dihydroxyacridone (**52**) was identified as a DNA topoisomerase II catalytic inhibitor [44, 58]. Experimental results suggested that the compound would act by stabilizing a ternary complex between this enzyme and the DNA. Once formed, this intermediate complex would be recognized as a cytotoxic lesion, leading to cellular death.

It is worth mentioning, that a later work reported that moving the chloro-substituent from the 7- to the 5-position (5-chloro-1,3-dihydroxyacridone) caused a loss of DNA topoisomerase II inhibitory activity, but it was observed that the 5-chloro congener was about 26-fold selective against HSV replication. Although the precise mechanism of action of this later compound was not defined, a blockade in DNA cleavage and packaging correlated with the inhibition of viral replication [59]. Thus, further optimization of the acridone lead and identification of the primary biochemical target(s) will be essential for the development of a viable antiviral acridone candidate from this group.

In other studies, a series of substituted triaryl heterocyclic compounds were tested against HSV-1 and 2, and assayed for inhibition of topoisomerase activity using a whole cell virus-induced cytopathogenic assay. The results indicated that the acridine analogs bearing substituted carboxamides and bulky 9-amino functionalities were able to inhibit herpesvirus infections as well as topoisomerase II relaxation of supercoiled DNA, most likely by blocking topoisomerase binding to DNA [38].

Moreover, CMA demonstrated direct non IFN-mediated antiviral *in vitro* activity against HADV type 6 [61]. The fact that CMA accumulates in cell nuclei rather than in the cytoplasm supports the idea that the nucleus is the main site of CMA activity [73]. Some observations indicated that CMA did not inhibit early HADV type 6 protein synthesis, but examination of infected cells showed altered structure of virus-specific inclusions and reduced yield of infectious virus. Therefore, it was proposed that the target point of CMA action would be the late DNA replication and/or packaging into virions during viral assembly, with the topoisomerase enzyme as principal candidate target for CMA.

On the other hand, in an extensive screening of anti-HIV-1 agents in chronically infected cell lines, it was found that 1-hydroxy-10-methyl-9(10*H*)-acridone (**18**) (RD6-5071) suppressed tumor necrosis factor (TNF)-α-induced HIV-1 expression and also inhibited phorbol 12-myristate 13-acetate (PMA)-induced HIV-1 expression. Interestingly, an

inhibition assay for protein kinase C (PKC), an enzyme involved in controlling the function of other proteins through their phosphorylation, revealed that RD6-5071 could reduce this enzyme activity. Thus, it was suggested that the acridone derivative suppressed HIV-1 replication at the transcriptional level primarily through a mechanism of PKC inhibition [62]. This target was also reported previously for 7-chloro-1,3-dihydroxyacridone (**52**) [74], but no further studies were communicated about this activity.

Finally, another acridone specific cellular target that has been studied is the inosine 5'-monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* synthesis of guanosine nucleotides [75]. This enzyme is a potential antiviral target, especially for RNA virus chemotherapy, as the adequate maintenance of nucleotide level is essential for normal cellular function, including the synthesis of DNA and RNA [76]. For that reason, it was believed that the inhibitory action of the derivative 10-allyl-6-chloro-4-methoxy-9(10*H*)-acridone (**36f**) observed against JUNV involved IMPDH as the main target. However, only a partial reversion of this acridone inhibitory effect was detected in the simultaneous presence of guanosine, with incomplete recovery of infective JUNV particles as well as viral RNA synthesis. Thus, the results indicated that IMPDH would be a possible cellular target for this compound, whereas another still unidentified target may also contribute to the antiviral activity of the acridone against arenaviruses [77].

4.2. Viral Targets

Regarding acridone viral targets, some important viral proteins have been identified and explored. On this basis, experiments designed to localize the mechanism of action for the prototype inhibitor citrusinine-I demonstrated an indirect blockade of HSV DNA and late protein synthesis. Interestingly, this antiviral effect was reversed by the addition of 2'-deoxynucleosides. According to this result, it was concluded that the viral ribonucleotide reductase would be the main target proposed for this compound, as inhibition of this enzyme would be responsible for the selective depletion of 2'-deoxynucleotides required for efficient viral DNA synthesis [55].

On the other hand, more reports describing acridone RNA viral targets were published. It was observed that temacrazine was able to inhibit acute HIV-1 infections and suppress the production of virus from chronically and latently infected cells. This compound exerted its mechanism of antiviral action through selective inhibition of HIV-1 transcription during the post-integrative phase of virus replication, without interfering with the transcription of cellular genes. It is worth noting that temacrazine, at nanomolar concentrations, inhibited the *in vitro* 3´ processing and strand transfer activities of HIV-1 integrase [37].

Furthermore, important flavivirus enzymes such as RNA helicase and RNA polymerase were also reported as direct targets for acridones. A group of *N*-substituted acridone-4-carboxamides were tested using a direct fluorometric helicase activity assay to determine its inhibitory properties against the HCV NS3 helicase and as putative transcription inhibitors in an *in vitro* transcription assay with the T7 RNA polymerase [29, 30]. A potent inhibitory activity towards both enzymes was also exhibited by derivatives with pyridine heterocyclic tailpieces using an HCV replicon system. Importantly, it was observed that this inhibition was independent of the enzyme concentration, suggesting lack of competition and interaction with the enzymes but was dependent on double-stranded RNA concentration, indicating interaction of the compounds with this substrate. In addition, the most active compound (a pyridylamide) had strong intercalatory properties. Compared to double-stranded DNA, RNA displays a greater diversity of folds and structures due to a variety of base pairing and other interactions between different regions of the single-stranded molecule, making this nucleic acid difficult to target using small molecules. Interestingly, it was found a strong preference for double-stranded RNA in the intercalating properties of this acridone-based HCV inhibitor [30]. However, the authors could not still elucidate if the compound affects RNA synthesis only through this intercalation, or if there is also a direct interaction of the compound with the viral enzymes or the combination of both effects.

Finally, other studies also using a subgenomic HCV replicon system demonstrated that another group of synthetic acridone derivatives had the ability to inhibit HCV replication [33], but none of these compounds showed NS3 helicase or NS5B polymerase inhibitory activity. Only the thiazolpiperazinyl derivative inhibited the helicase activity but its anti-enzymatic activity was approximately 10 times less than the anti-HCV activity observed in the cellular assay.

5. CONCLUSIONS AND PERSPECTIVES

Acridones represent a group of compounds with variable chemical substitutions which have been studied for several decades as candidates for biomedical application due to the wide range of their biological properties. In the field of natural products, new acridonic derivatives with different biological activities are periodically isolated. Therefore, several research groups are working in isolation, purification and characterization of alkaloids from various biological sources. Furthermore, significant efforts have focused on synthesis of new members of this family under less forceful conditions. Particularly, the use of alternative energy sources such as microwave irradiation, the application of ultrasound probes, and the discovery of unusual reactive combinations through a one-pot process allowed the synthesis of a variety of new derivatives.

Concerning antiviral activity, the initial focus was on DNA viruses, particularly herpesviruses, based on the potential of virus inhibition by targeting cellular enzymes involved in DNA replication. Several attempts were intended to obtain a selective virus inhibition through modification of the chemical structure of the acridone molecule by addition of different active groups. Although the methods of synthesis were improved, results were not fully successful and specificity of the derivatives against herpesvirus multiplication without significantly affecting the host cell could not be achieved. The diverse biochemical actions attributed to certain active anti-HSV acridones were considered a confounding issue for target identification leading to failure in identification and differentiation between viral- and non viral drug interactions in infected cells [78].

In recent years, the perspectives of acridones as antiviral agents regained interest because, in contrast to the situation described for herpesviruses, different subclasses of acridones were synthesized and found to be selective inhibitors of RNA viruses that represent a serious hazard for public health given the lack of chemotherapy. The multiplication of hepatitis C virus, dengue virus and hemorrhagic fever viruses was found susceptible to acridones, with selectivity indexes higher than 200. Mechanistic studies are indicating that inhibition is related to inhibition of RNA replication or transcription involving probably cellular and viral components participating in these processes, but without affecting cell viability. Although a wide spectrum of antiviral effectiveness is exhibited, certain chemotypes, like the *N*-allyl substituted acridones, which are active against flaviviruses and arenaviruses, warrants further investigation for optimization and development of acridone-based agents with potential for clinical therapeutic application.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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