Inhibition of Subgenomic Hepatitis C Virus RNA Replication by Acridone Derivatives: Identification of an NS3 Helicase Inhibitor

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Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Via del Liceo, 1, 06123 Perugia, Italy, Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium, Istituto di Genetica Molecolare, CNR-Pavia, Via Abbiategrasso, 207, 27100 Pavia, Italy, and Dipartimento di Scienze Farmaceutiche, Università di Padova, Via Marzolo 5, 35131, Padova, Italy

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We report the synthesis and structure−activity relationship (SAR) of a large series of acridones and acridone-fragment derivatives designed on the basis of the selective antihepatitis C virus (HCV) activity shown by acridone **2**, previously studied as a potential antibovine viral diarrhea virus (BVDV) compound. The evaluation of their ability to inhibit the HCV replication in Huh-5-2 cells led to the identification of new, selective inhibitors. This indicates that the acridone skeleton, when properly functionalized, is a suitable scaffold to obtain potential anti-HCV agents. Interestingly, during identification of possible cellular and viral targets, it was discovered that compound **23** exerts inhibitory activity on the HCV NS3 helicase, a very promising target for the development of anti-HCV drugs.

Introduction

Worldwide, an estimated 170 million people are chronically infected with hepatitis C virus (HCV[a]) and are at high risk for developing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.[1] Current treatment consists of a combination of subcutaneously administrated pegylated interferon-α with the orally dosed nucleoside analogue ribavirin. This combination is generally poorly tolerated, is contraindicated for many patients, and is only effective in controlling the disease in a fraction of the individuals who are eligible for therapy.[2] Therefore, there is an obvious, pressing need to develop more effective and tolerated treatments. Today, a number of molecules are being studied in clinical trials,[3] but none of them have yet been approved.

In an earlier paper,[4] we reported the design, synthesis, and antiviral evaluation of a small series of acridone derivatives (compounds **1**−**6**, Table 1), identifying some selective inhibitors of bovine viral diarrhea virus (BVDV, a virus related to HCV[5,6]) replication. 1,3-Dihydroxy derivative **1**, characterized by an amino group at the C-7 position and the 2-pyridinylpiperazine at the C-6 position, emerged as the most interesting compound displaying anti-BVDV activity (EC_{50}) of 0.5 ± 0.2 μg/mL with no cytotoxicity at a concentration 200 times higher (CC_{50} > 100 μg/mL).

As part of our efforts to identify new anti-HCV agents, acridones **1**−**6** have now been evaluated in the HCV genotype 1 subgenomic replicon system. The biological results reported in Table 1 revealed an interesting antiviral activity for some of the derivatives, even if no strict correlation was observed between the anti-BVDV and anti-HCV activity. In particular, compound **1** exhibited an anti-HCV activity that was comparable to that for BVDV but proved to be somewhat more toxic against the human hepatoma Huh-5-2 cells than on the bovine kidney cells. On the other hand, the 1,3-dimethoxy analogue **2**, which was completely inactive against BVDV, proved to be equally potent in the replicon assay, but above all, it was the most selective, being devoid of any toxicity. In several independent experiments, this compound was shown to inhibit viral RNA synthesis in a dose-dependent manner (Figure 1). This interesting biological profile made **2** a valid hit compound worthy of an in-depth investigation aimed at identifying the structural pharmacophoric requirements and increasing the HCV replicon inhibitory activity while maintaining the selectivity. Here we describe the design, synthesis, and structure−activity relationship (SAR) of an enlarged series of acridone derivatives (**7**−**42** Table 2) obtained by modifying the substitution pattern on the acridone nucleus. To explore the structural features responsible for the selective anti-HCV activity of **2**, more incisive modifications were made on the acridone scaffold itself, giving rise to derivatives **43**−**48** (Figure 2). Various viral and cellular proteins were considered as the target for this class of molecules; HCV NS3 helicase was the only enzyme that was found to be inhibited by our compounds, albeit with moderate potency.

Chemistry

Acridone derivatives **7**−**11** variously functionalized at the C-ring were synthesized as reported in Scheme 1, analogous to our previously reported analogues.[4] Briefly, an Ullman reaction of 2,4-dichloro-5-nitrobenzoic acid[7] with various anilines gave diphenylamine carboxylic acids **49**, **50**, **51**,[8] and **52**, which were then cyclized using PPA at 120 °C to give the nitroacridones **53**−**57**. The ring closure of monomethoxy anthranylate **49** led to the formation of two regioisomers, **53** and **54**; the mixture was difficult to separate, so it was used in the next step and then separated. The successive sequential N-methylation to **58**−**60**, **61**,[9] and **62**, nucleophilic reaction with 1-(2-pyridinyl)piperazine to **63**−**67**, and nitro reduction afforded the target compounds **7**−**11**. With few exceptions, $SnCl_2 \cdot 2H_2O$ under acid

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[a] Abbreviations: HCV, hepatitis C virus; BVDV, bovine viral diarrhea virus; SAR, structure−activity relationship; PPA, polyphosphoric acid; IMPDH, inosine monophosphate dehydrogenase; MTS/PMS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium)/phenazinemethosulfate.

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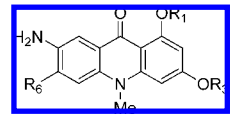
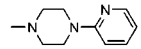
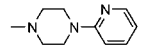
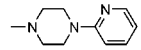
Table 1. Structure, Anti-BVDV Activity, Anti-HCV Activity, and Cytoxic/Cytostatic Effect of Reference Compounds



				BVDV assay[b]		HCV replicon assay	
compd[a]	R_1	R_3	R_6	EC_{50} (ug/ml)	CC_{50} (ug/ml)	EC_{50}[c] (ug/ml)	CC_{50}[d] (ug/ml)
1	H	H		0.5 ± 0.2	> 100	1.7 ± 0.2	21 ± 3
2	Me	Me		> 100	> 100	1.6 ± 0.4	50
3	H	Me		4 ± 2	> 100	13 ± 0.3	> 50
4	H	H	Cl	0.4 ± 0.2	5.0 ± 0.7	1.48 ± 0.01	3 ± 0.03
5	Me	Me	Cl	> 100	> 100	20	> 50
6	H	Me	Cl	0.7 ± 0.3	> 33	14 ± 11	41 ± 13

[a] Reference 4. [b] Data from ref 4. [c] EC_{50} values were obtained as described in the Experimental Section. All the data represent mean values for at least three independent experiments ± standard deviation. [d] CC_{50} values were obtained as described in the Experimental Section. All the data represent mean values for at least three independent experiments ± standard deviation.

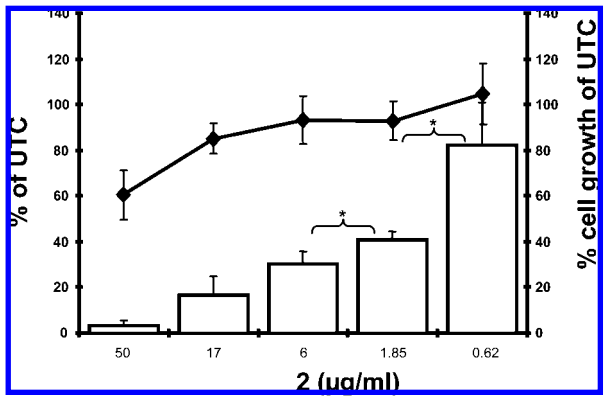


Figure 1. Effect of **2** on HCV replicon replication in Huh-5-2 cells (measured as luciferase signal, bars) and on the proliferation of exponentially growing cells (diamonds). Data are expressed as percentage of untreated controls (UTC) and are mean values ± SD for four independent experiments: (*) $P < 0.05$.

condition was used as a general procedure to reduce the nitro group, even if the aminoderivative was obtained in very low yield mainly due to the formation of the side *o*-chloroamino derivative. It is known that $SnCl_2$ can promote the insertion of a chlorine atom at the *o*-position of anilines;[10,11] however, the very low solubility of the nitroacridones in the common solvents did not permit the use of other reduction methods.

Following the synthetic pathway described above, the reaction of 2-chloro-4,5-difluorobenzoic acid with 3,5-dimethoxyaniline (not shown) gave 7-fluoroacridone **33** (Table 2).

Acridone derivatives **12**−**15** (Scheme 2) were prepared by O-alkylation with the selected alkyl halide of the 1-hydroxy-7-nitro derivative **69**,[4] in turn conveniently prepared by selective de-O-methylation of **68**[4] with LiCl, followed by catalytic reduction of nitro intermediates **70**−**73**.

Scheme 3 depicts the synthesis of the 7-amino-1,3-dimethoxy derivatives **16**−**26** variously functionalized at the N-10 or C-6 position of the acridone nucleus. The target compounds **16**−**18** were prepared by reacting the corresponding precursors **74**,[12] **76**,[12] and **77**, the latter prepared by alkylation of **74**[12] with 1-iodo-3-methylbutane, with 1-(2-pyridinyl)piperazine followed by reduction of the nitro group with $SnCl_2$. Following the same procedure, *N*-methyl acridones **19**−**26** were also prepared by reacting precursor **75**[4] with selected 4-arylpiperazines or with NaOMe for **26**.

The 1,3-dimethoxyacridones **22**, **24**, and **26** were converted to the corresponding 1-hydroxy-3-methoxy (**27**, **28**, and **29**) and 1,3-dihydroxy derivatives (**30**, **31**, and **32**) using 48% HBr in the presence of traces of AcOH. The progress of the reaction was strictly monitored to avoid the formation of the sole dihydroxy derivatives.

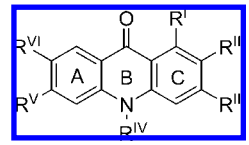
N-Isopentyl derivative **39** was prepared by reducing acridone **77** (Scheme 3). The N-10 unsubstituted C-6 chlorine derivatives **40**−**42** were directly prepared starting from precursor **74** through reduction (to **40**) and successive de-O-methylation (to **41** and **42**) (Scheme 3).

Target compounds **34** and **35** (Table 2) were both prepared (not shown) starting from the hit compound **2**.[4] In particular, *N,N*-dimethylamino derivative **34** was synthesized by methylation of the C-7 amino group with a large excess of MeI in the presence of K_2CO_3. The C-7 unsubstituted derivative **35** was prepared by converting the amino group into the diazonium salt and then decomposed in the presence of 50% H_3PO_2 aqueous solution.

Diphenylamine **43** was synthesized as shown in Scheme 4 by closely following the procedure illustrated for the synthesis of acridones **7**−**11**, bypassing the cyclization step. Thus, antranilic acid **78**[12] was N-methylated, the obtained methyl ester **79** was coupled with 1-(2-pyridinyl)piperazine to form intermediate **80**, which was then reduced to the corresponding amino derivative **81** and finally hydrolyzed in mild basic condition to the target compound **43**.

For the synthesis of diphenylamine **44**, the route depicted in Scheme 5 was employed. Starting with 2,4-difluoronitrobenzene, there were two nucleophilic displacements in succession: the first with 1-(2-pyridinyl)piperazine, using toluene as solvent, to give ortho-substituted **82** (assigned by NOESY experiments) as the major regioisomer, the second with 3,5-dimethoxy-*N*-

Table 2. Anti-HCV Activity and Cytostatic Effect of Acridone Derivatives



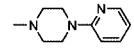
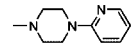
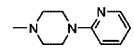
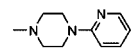
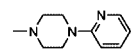
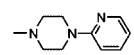
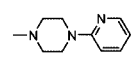
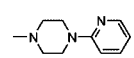
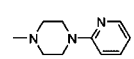
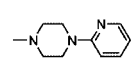
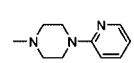
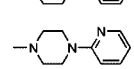
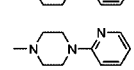
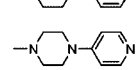
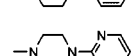
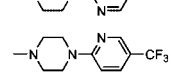
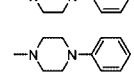
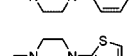
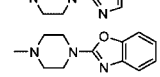
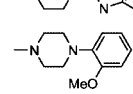
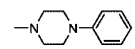
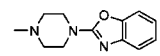
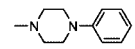
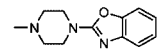
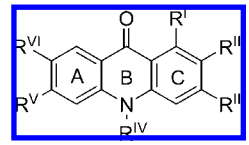
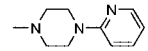
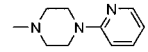
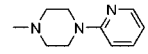
compd	R^I	R^{II}	R^{III}	R^{IV}	R^V	R^{VI}	EC_{50}[a] (ug/mL)	CC_{50}[b] (ug/mL)
2[c]	OMe	H	OMe	Me		NH_2	1.6 ± 0.4	50
7	H	H	OMe	Me		NH_2	0.8[d]	11
8	OMe	H	H	Me		NH_2	12.5	28.5
9	H	OMe	OMe	Me		NH_2	6[d]	14
10	H	H	H	Me		NH_2	4[d]	29
11	Me	H	Me	Me		NH_2	5	> 50
12	OEt	H	OMe	Me		NH_2	4	> 50
13	OPr	H	OMe	Me		NH_2	17	> 50
14	O*i*-Bu	H	OMe	Me		NH_2	3.8	27
15	OCH_2CO_2H	H	OMe	Me		NH_2	35[d]	> 50
16	OMe	H	OMe	H		NH_2	4[d]	19
17	OMe	H	OMe	Et		NH_2	4[d]	11
18	OMe	H	OMe	*i*-Pe		NH_2	10[d]	10
19	OMe	H	OMe	Me		NH_2	5.5	> 50
20	OMe	H	OMe	Me		NH_2	> 50	> 50
21	OMe	H	OMe	Me		NH_2	> 50	> 50
22	OMe	H	OMe	Me		NH_2	9 ± 10	34
23	OMe	H	OMe	Me		NH_2	3 ± 2	> 50
24	OMe	H	OMe	Me		NH_2	27	> 50
25	OMe	H	OMe	Me		NH_2	16	> 50
26	OMe	H	OMe	Me	OMe	NH_2	9[d]	> 50
27	OH	H	OMe	Me		NH_2	2.03	8.5
28	OH	H	OMe	Me		NH_2	50	> 50
29	OH	H	OMe	Me	OMe	NH_2	18 ± 15	> 50
30	OH	H	OH	Me		NH_2	4.4	16.2
31	OH	H	OH	Me		NH_2	17	> 50
32	OH	H	OH	Me	OMe	NH_2	5	26

Table 2. Continued



compd	R^I	R^{II}	R^{III}	R^{IV}	R^V	R^{VI}	EC_{50}[a] (ug/ml)	CC_{50}[b] (ug/ml)
33	OMe	H	OMe	Me		F	> 50	> 50
34	OMe	H	OMe	Me		$N(Me)_2$	1[d]	3
35	OMe	H	OMe	Me		H	2.3 ± 0.4	> 50
36[e]	OMe	H	OMe	Et	Cl	NH_2	5	14
37[e]	OH	H	OMe	Et	Cl	NH_2	4.5	> 50
38[e]	OH	H	OH	Et	Cl	NH_2	5	> 50
39	OMe	H	OMe	*i*-Pe	Cl	NH_2	> 50	> 50
40	OMe	H	OMe	H	Cl	NH_2	5.7	13
41	OH	H	OMe	H	Cl	NH_2	8 ± 3	> 50
42	OH	H	OH	H	Cl	NH_2	19	25

[a] EC_{50} values were obtained as described in the Experimental Section and are shown as mean values of two to three determinations. [b] CC_{50} values were obtained as described in the Experimental Section and are shown as mean values of two to three determinations. [c] Reference 4. [d] EC_{50} and CC_{50} are shown as single determination. [e] Reference 12.

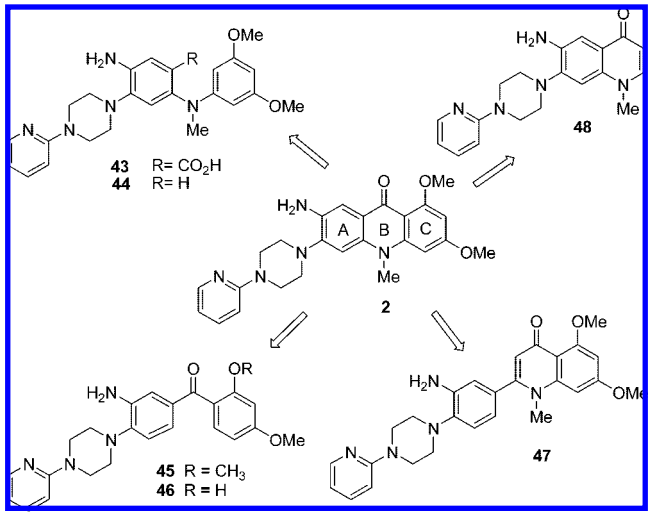


Figure 2. Acridone scaffold disconnection.

methylaniline[13] in the presence of *t*-BuOK to give diphenylamine intermediate **83** which was finally reduced to **44**.

In an initial attempt to obtain benzophenone **45** (Scheme 6), a Friedel−Crafts reaction of 4-chloro-3-nitrobenzoyl chloride was carried out with 1,3-dimethoxybenzene, but under the usual conditions ($AlCl_3/CH_2Cl_2$ at room temperature or reflux; $AlCl_3$/nitrobenzene at reflux; $FeCl_3/Bi_2O_3$ in CH_2Cl_2 at room temperature or reflux) the expected dimethoxybenzophenone was not obtained. However, when the reaction was carried out using microwaves and $AlCl_3$ as catalyst, according to a recent publication,[14] compound **84** was obtained in 65% yield. The reaction conditions also caused a simultaneous de-O-methylation which occurred at the C-2 position as expected and confirmed by NOESY experiments. The successive nucleophilic reaction with 1-(2-pyridinyl)piperazine followed by catalytic reduction gave monomethoxy derivative **46** through the intermediate **85**. The O-methylation of intermediate **85** gave **86** which was catalytically reduced to the desired dimethoxy derivative **45**.

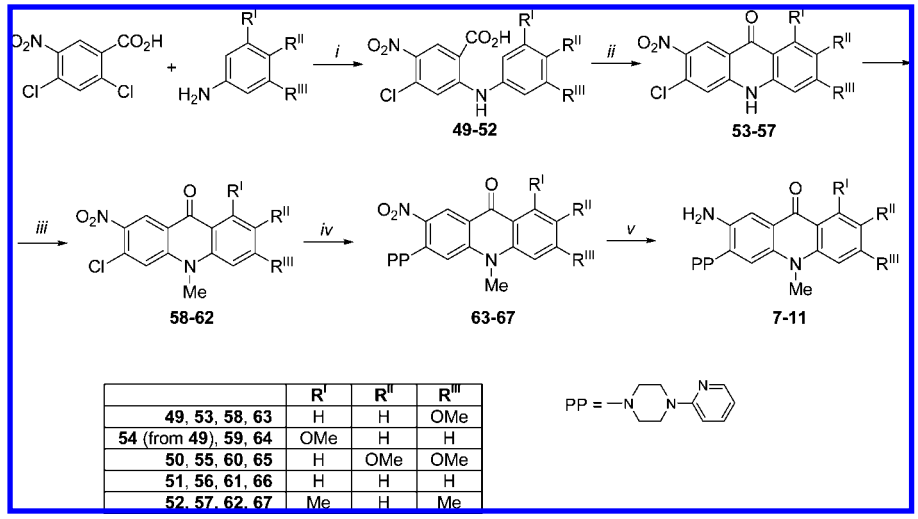
Phenylquinolone **47** (Scheme 7) was synthesized by starting with nitro intermediate **87**, prepared as previously reported by us,[15] which was coupled with 1-(2-pyridinyl)piperazine to give **88** and then reduced under catalytic conditions.

Results and Discussion

All the target compounds synthesized in this study were evaluated for their ability to inhibit HCV replication in Huh-5-2 cells, a Huh-7 derived cell line containing a genotype 1b HCV replicon with the luciferase report gene. INF-α was included as a positive control. The cytostatic potential of the compounds was evaluated in parallel in the same cell line. Data are summarized in Tables 2 and 3.

With selection of acridone **2** as the hit compound, about 40 analogues were synthesized. Of these, derivatives **12**, **14**, **19**, **23**, and **35** were found to selectively inhibit HCV RNA synthesis in the context of the intact cell, in a dose-dependent manner. Other compounds proved to be cytostatic or did not inhibit the

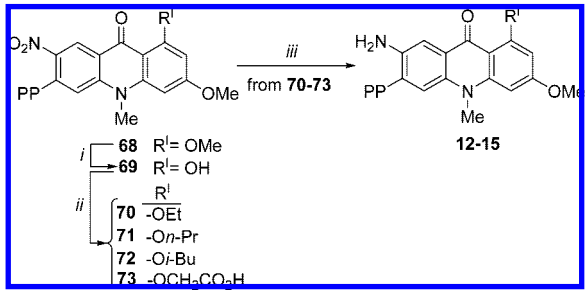
Scheme 1[a]



	R^I	R^{II}	R^{III}
49, **53**, **58**, **63**	H	H	OMe
54 (from **49**), **59**, **64**	OMe	H	H
50, **55**, **60**, **65**	H	OMe	OMe
51, **56**, **61**, **66**	H	H	H
52, **57**, **62**, **67**	Me	H	Me

[a] Reagents and conditions: (i) $Cu(OAc)_2 \cdot 2H_2O$, KOAc, Et_3N, 2-propanol, reflux; (ii) PPA, 120 °C; (iii) MeI, K_2CO_3, dry DMF, 90 °C; (iv) 1-(2-pyridinyl)piperazine, dry DMF, 90 °C; (v) $SnCl_2 \cdot 2H_2O$, 8 N HCl, reflux.

Scheme 2[a]



[a] Reagents and conditions: (i) LiCl, DMF, reflux; (ii) RX, Cs_2CO_3, dry DMF, 90 °C; (iii) H_2, Raney Ni, DMF, 1 atm, room temp. For R^I of the target compounds, see Table 2.

replication of the replicon. Some insights in the SAR were derived from this data set. Regarding the modifications made on the C-ring, both monomethoxy derivatives **7** and **8**, derivative **9** bearing vicinal R^{II} and R^{III} methoxy groups, and derivative **10** which lacks both methoxy groups proved to be more toxic than the parent compound **2** without appreciable selective inhibition of HCV subgenomic replication. On the other hand, the presence of R^I and R^{III} methyl groups gave compound **11** whose activity was comparable to that of **2** but without a dose-dependent effect. The R^I substituent was further studied by replacing the methoxy group with longer side chains such as ethoxy (**12**), propoxy (**13**), and isobutyloxy (**14**) as well as with an acetic side chain (**15**). The antiviral activity was maintained by derivatives **12** and **14**, although the latter was somewhat more cytostatic.

Overall, the above data show that the coupled presence of R^I and R^{III} methoxy groups is the best substitution pattern for the C-ring. The biological profile of a few analogues bearing the R^I hydroxyl group (**27** and **28**) or R^I and R^{III} hydroxyl groups (**30** and **31**) also supports this observation.

Regarding the R^{IV} substituent, the methyl group present in the hit compound **2** was confirmed to be the best one and cannot be omitted as in compound **16** or replaced with ethyl (**17**) or isopentyl group (**18**), as this results in an increase in cytostatic effect.

Besides 1-(2-pyridinyl)piperazine, which characterizes **2**, its regioisomer 1-(4-pyridinyl)piperazine, as well as the 1-(1,3-thiazol-2-yl)piperazine, can also be a suitable R^V side chain resulting in compounds **19** and **23** which are endowed with an antiviral activity comparable to that of the reference compound. All the other 4-arylpiperazines (derivatives **20**, **21**, **22**, **24**, and **25**) were unsuitable as R^V substituent. When a methoxy group was employed as R^V substituent (compounds **26**, **29**, and **32**), a slight decrease in antiviral activity was observed without a dose-dependent effect.

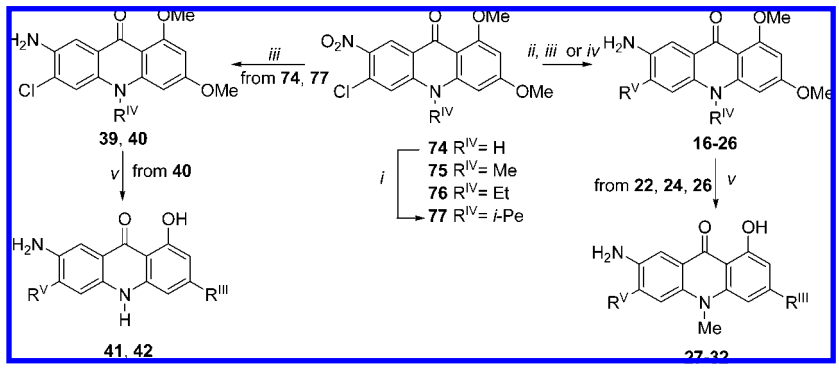
Good anti-HCV activity was shown by R^V chloro derivatives **36**, **37**, and **38** (Table 2), previously prepared by us as potential antitumoral agents.[12] Besides having a chlorine atom as an R^V substituent instead of the pyridinylpiperazine, compounds **36**−**38** differ from **2** bearing an R^{IV} ethyl group. When the chlorine atom was coupled with an R^{IV} methyl group (see derivatives **4**−**6**[4] in Table 1), contrasting anti-HCV results were obtained. Therefore, to further explore the role of the R^{IV} substituent in the chloroacridone series, new analogues were prepared (compounds **39**−**42**). Unfortunately this novel set of data did not contribute to the delineation of an SAR and discouraged the synthesis of further analogues.

The deletion of the R^{VI} amino group from compound **2** was fruitful; in fact, compound **35** emerged as one of the best molecules synthesized in this study. On the contrary, when the amino group was replaced by a fluorine atom, there was a loss of activity (compound **33**) while its N,N-alkylation (compound **34**) greatly increased the cytostatic effect.

Regarding the incisive structural modifications made on the acridone scaffold itself (Figure 2), the opening of the B ring to produce diphenylamines was detrimental; **43** was inactive while **44** proved highly cytostatic (Table 3). An increase in toxicity was also noted in benzophenone **45**, derived from a different acridone skeleton opening; however, the C-2 de-O-methylated derivative **46** resulted in appreciable anti-HCV activity (EC_{50} = 3.7 μg/mL) and was not cytostatic. The 2-phenylquinolone **47**, derived from the shifting of the A ring to the C-2 position of the dimethoxyquinolone nucleus, was neither active nor toxic, while a weak antiviral activity (EC_{50} = 11 μg/mL) and no cytostatic activity were displayed by quinolone derivative **48**,[16] in which the acridone structure was further simplified by the deletion of the C ring.

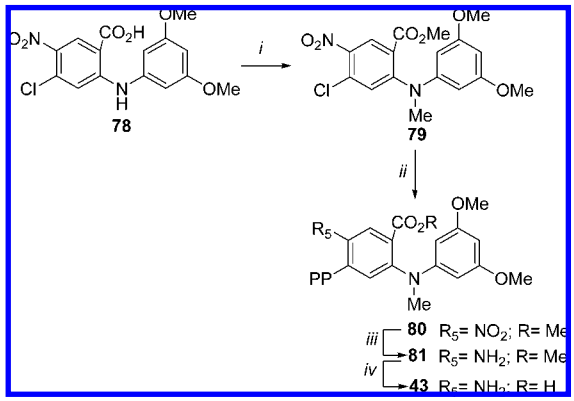
The acridone moiety is thus the most suitable for granting selective antiviral activity, but the benzophenone scaffold, when

Scheme 3[a]



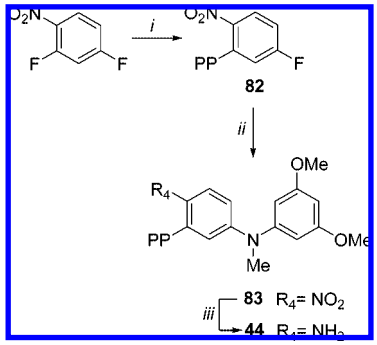
[a] Reagents and conditions: (i) 1-iodo-3-methylbutane, K_2CO_3, dry DMF, 90 °C; (ii) R^VH, dry DMF, 90 °C (for **26**, MeONa, dry DMF); (iii) $SnCl_2 \cdot 2H_2O$, 8 N HCl, reflux; (iv) H_2, Raney Ni, DMF, 1 atm, room temp; (v) 48% HBr, AcOH, reflux. For R^{III}, R^{IV}, and R^V, see Table 2.

Scheme 4[a]



[a] Reagents and conditions: (i) MeI, K_2CO_3, dry DMF, 90 °C; (ii) 1-(2-pyridinyl)piperazine, dry DMF, 90 °C; (iii) H_2, Raney Ni, EtOAc, 1 atm, room temp; (iv) $LiOH \cdot 2H_2O$, dioxane/H_2O (3:1).

Scheme 5[a]

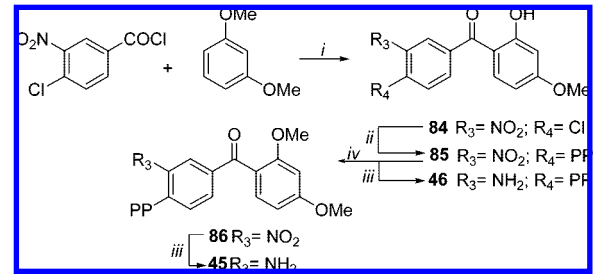


[a] Reagents and conditions: (i) 1-(2-pyridinyl)piperazine, Et_3N, toluene; (ii) 3,5-dimethoxy-*N*-methylaniline, *t*-BuOK, DMSO, 0 °C; (iii) H_2, Raney Ni, EtOAc, 1 atm, room temp.

properly functionalized as in compound **46**, is also a valid hit in an effort to obtain new anti-HCV agents.

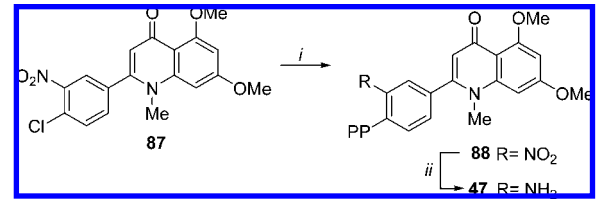
In summary, the structural modifications made on the hit compound **2** yielded new analogues including **12**, **14**, **19**, **23**, and **35** which inhibited HCV RNA replication in a selective and dose-dependent manner. However, in order for these new chemotypes to be developed as anti-HCV agents, their potency and selectivity must be increased. The identification of their molecular target could help the optimization of this class of HCV replication inhibitors. Therefore, the effect of the compounds on a number of potential cellular and viral targets was investigated.

Scheme 6[a]



[a] Reagents and conditions: (i) $AlCl_3$, microwaves, 57% potency, 7 min; (ii) 1-(2-pyridinyl)piperazine, dry DMF, 90 °C; (iii) H_2, Raney Ni, DMF, 1 atm, room temp; (iv) MeI, Cs_2CO_3, dry DMF, 90 °C.

Scheme 7[a]



[a] Reagents and conditions: (i) 1-(2-pyridinyl)piperazine, dry DMF, 90 °C; (ii) H_2, Raney Ni, DMF, 1 atm, room temp.

Table 3. Anti-HCV and Cytostatic Effect of the Molecules Synthesized by Disconnecting the Acridone Scaffold

compd	EC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
43	>50	>50
44	4.1	7.2
45	6.6	15
46	3.7	>50
47	>50	>50
48[c]	11	>33

[a] EC_{50} values were obtained as described in the Experimental Section and are shown as single determination. [b] CC_{50} values were obtained as described in the Experimental Section and are shown as single determination. [c] Reference 16.

Acridone-based compounds have recently been reported to be potent reversible and uncompetitive inosine monophosphate dehydrogenase (IMPDH) inhibitors[17] that are effective on a rat adjuvant arthritis model. IMPDH is recognized as being one of the potential cellular targets in anti-HCV therapy.[18,19] Ribavirin exerts its antiviral activity against many viruses by inhibiting (as its 5′-monophosphate) this enzyme,[20,21] similar to the uncompetitive IMPDH inhibitor mycophenolic acid[21−23] and the Vertex Pharmaceutical compound VX-497.[21,24] The latter was not further developed

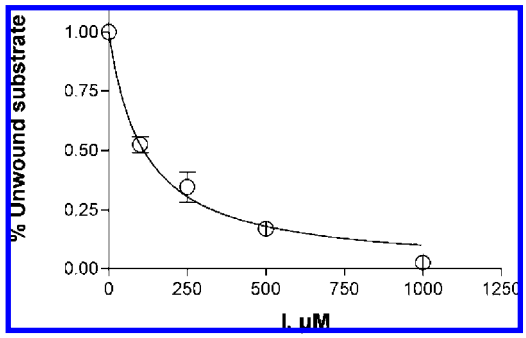


Figure 3. Dose−response curve for the inhibition of NS3 helicase activity by compound **23**. Values are the mean of three independent replicates. Error bars are ±SD. Data were fitted to the equation (% obs) = (% max)/(1 + [I]/IC_{50}), where (% obs) is the observed percentage of unwound substrate, (% max) is the maximal unwinding percentage in the absence of the inhibitor, [I] is the inhibitor concentration, and IC_{50} is the 50% inhibitory dose.

as an HCV inhibitor.[3] To determine if IMPDH was the target of the acridones reported in this study, the cytostatic effect in Vero cells alone and in combination with guanosine was tested for the best molecules (**2**, **14**, **19**, **23**, and **35**). There was no evidence that the cytostatic effect was reversed in the presence of guanosine (data not shown).

When the activity of the same molecules was tested on the HCV RNA polymerase NS5B, one of the most relevant key enzyme of the HCV replication, none of the compounds showed inhibitory activity at concentrations up to 100 μM. These data are in agreement with what we previously reported for acridone **1**, which had little or no inhibitory effect on the BVDV NS5B polymerase activity.[4]

None of the above compounds were able to inhibit the NS3 NTPase activity (IC_{50} > 1 mM). However, as shown in Figure 3, the thiazolpiperazinyl derivative **23** inhibited the helicase activity with an IC_{50} of 50 ± 5 μg/mL (110 ± 12 μM). Its antienzymatic activity was approximately 10 times less than the anti-HCV activity in the cellular assay. However, such discrepancies are not uncommon because of the differences between the in vitro conditions (for example, high enzyme-to-substrate ratio) and the replicon assay. Thus, so far, our data support the reasonable hypothesis that the target of this compound is likely the HCV NS3 helicase. NS3 has long been regarded as a very promising target for the development of anti-HCV drugs. It is an essential enzyme for viral replication and bears little homology to cellular helicases. However, to date, only a few examples of inhibitors of this activity have been reported, particularly in the non-nucleoside class.[25] Interestingly, a series of compounds strictly related to our acridones have recently been reported to be good inhibitors of HCV helicase activity.[26] Although for these compounds the inhibitory effect in the enzymatic assay did not translate into antiviral activity in the replicon assay, this study lends support to our observation that acridones may have the potential to inhibit HCV replication by targeting the helicase.

Further studies are in progress to determine the precise mechanism by which **23** inhibits the NS3 helicase activity. In particular, we are addressing whether the inhibition is due to direct enzyme−drug interaction or is mediated by the compound binding to the nucleic acid substrate.

Finally, since some of the known antiviral acridone derivatives are able to inhibit the topoisomerase II enzyme,[27,28] we wanted to ascertain whether this enzyme was recognized by the compounds reported here. Therefore, topoisomerase II unknotting and relaxation assays were performed for hit compound **2** along with derivatives **1**[4] and **4**[4] which are characterized by a different degree of toxicity. Doxorubicin and *m*-amsacrine (*m*-AMSA), respectively, were included as positive controls in the two assays. From the analyses of the gel (data not shown) it appeared that the tested acridones were inactive against the human enzyme, indicating that their cytostatic effect in Huh-5-2 cells was not dependent on the topoisomerase II interaction.

Conclusions

Starting from hit compound **2**, a large series of acridones and acridone-fragment derivatives were synthesized and evaluated for their ability to inhibit the HCV replication in Huh-5-2 cells. Some new selective anti-HCV compounds were identified revealing that the acridone skeleton, when properly functionalized, represents a suitable scaffold for obtaining potential anti-HCV agents. Structure features responsible for the HCV replication inhibition are a methoxy, ethoxy, or isobutyloxy group as R^I substituent, a 4-arylpiperazine as R^V side chain with 1-(2-pyridinyl)-, 1-(4-pyridinyl)-, and 1-(1,3-thiazol-2-yl)piperazine which confer the best activity, a R^{IV} methyl group, and an amino group or hydrogen atom as an R^{VI} substituent. Searching for their molecular target, we have identified the thiazolpiperazinyl derivative **23** which inhibited, albeit with low potency, the HCV NS3 helicase. Studies are in progress to gain insight into the precise mechanism of action of **23**, which would ultimately provide a structural basis for the discovery of improved anti-NS3 helicase acridones.

For all the other selective acridones identified in this study, which did not recognize the NS3 helicase, the anti-HCV replicon activity could be due to either a different unknown mechanism of action or a polypharmacological effect. The latter interpretation is in line with what has been previously reported by Bastow[29] for other antiviral acridones, for which more than one biochemical target has been identified.

Experimental Section

All reactions were routinely checked by thin-layer chromatography (TLC) on silica gel $60F_{254}$ (Merck) and visualized by using UV or iodine. Column chromatography separations were carried out on Merck silica gel 60 (mesh 70−230), flash chromatography on Merck silica gel 60 (mesh 230−400), and reverse phase chromatography on Merck RP C_{18} (40−63 μm, Lichroprep). Melting points were determined in capillary tubes (Büchi Electrotermal model 9100) and are uncorrected. Elemental analyses were performed on a Carlo Erba elemental analyzer, model 1106, and data for C, H, and N are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded at 200 MHz (Bruker Avance DPX-200), while ^{1}H−^{1}H NMR NOESY spectra were recorded at 400 MHz (Bruker Avance DRX-400) using residual solvent such as chloroform (δ = 7.26) or dimethyl sulfoxide (δ = 2.48) as an internal standard. The 2D NOESY experiments were run in phase-sensitive mode at 298 K. Data processing was performed with standard Bruker software XwinNMR. Chemical shifts are given in ppm (δ), and the spectral data are consistent with the assigned structures. GC/MS analyses were carried out with an HP 6890 gas chromatograph (25 m dimethylsilicone capillary column) equipped with an HP 5973 mass selective detector. Microwave reactions were carried out using a domestic unmodified microwave oven (Daewoo KOR-63F7, 2.45 MHz, 700 W). Reagents and solvents were purchased from common commercial suppliers and were used as such. After extraction, organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated with a Büchi rotary evaporator at reduced pressure. Yields were of purified product and were not

optimized. All starting materials were commercially available, unless otherwise indicated.

General Procedure for Ullman Reaction. 4-Chloro-2-[(3,4-dimethoxyphenyl)amino]-5-nitrobenzoic Acid (50). A mixture of 2,4-dichloro-5-nitrobenzoic acid[7] (5.00 g, 21.20 mmol), 3,4-dimethoxyaniline (4.87 g, 31.80 mmol), KOAc (6.24 g, 63.60 mmol), $Cu(OAc)_2 \cdot 2H_2O$ (0.13 g, 0.64 mmol), and Et_3N (8.92 mL, 63.60 mmol) in 2-propanol (150 mL) was refluxed for 12 h. The solvent was then concentrated under reduced pressure, the mixture was poured into ice−water, acidified with 2 N HCl to pH ∼3, and the solid formed was filtered, collected, and dissolved in $CHCl_3$. The organic solution was extracted with water and evaporated to dryness. The solid obtained was triturated with EtOH and filtered to give **50** (4.0 g, 54%) as a brown solid: mp 248−249 °C; ^{1}H NMR (DMSO-d_6) δ 3.70 and 3.80 (s, each 3H, OCH_3), 6.80−7.10 (m, 4H, aromatic CH), 8.70 (s, 1H, H-6), 10.10 (s, 1H, NH), 14.00 (bs, 1H, CO_2H).

General Procedure for Cyclization Reaction. 3-Chloro-6,7-dimethoxy-2-nitro-9(10*H*)-acridinone (55). A mixture of **50** (2.50 g, 7.09 mmol) and PPA (12.5 g) was heated in an oil bath at 120 °C for 7 h. After cooling, the mixture was triturated with ice−water and neutralized with 10% NaOH, and the dark precipitate was collected by filtration and then recrystallized by DMF to give compound **55** (0.5 g, 21%) as a yellowish solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 and 3.94 (s, each 3H, OCH_3), 6.90 (s, 1H, H-4), 7.48 and 7.52 (s, each 1H, H-5 and H-8), 8.75 (s, 1H, H-1), 12.10 (s, 1H, NH).

General Procedure for N-Alkylation Reaction. 3-Chloro-6,7-dimethoxy-10-methyl-2-nitro-9(10*H*)-acridinone (60). MeI (0.33 mL, 5.37 mmol) was added to a mixture of compound **55** (0.45 g, 1.34 mmol) and K_2CO_3 (0.74 g, 5.37 mmol) in dry DMF (15 mL). The reaction mixture was heated at 90 °C for 4 h. After cooling, the mixture was poured into ice−water and the solid obtained was filtered, washed with water, and recrystallized by EtOH/DMF to give compound **60** (0.35 g, 74%) as a brown solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 and 3.83 (s, each 3H, OCH_3) 3.90 (s, 3H, NCH_3), 7.09 (s, 1H, H-4), 7.41 (s, 1H, H-5), 8.00 (s, 1H, H-8), 8.70 (s, 1H, H-1).

General Procedure for Coupling Reaction. 2,3-Dimethoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (65). A mixture of compound **60** (0.31 g, 0.88 mmol) and 1-(2-pyridinyl)piperazine (0.4 mL, 2.64 mmol) in dry DMF (10 mL) was heated at 90 °C for 7 h. The solution was concentrated under reduced pressure, and after the mixture was cooled, the precipitate was collected by filtration, washed with EtOH, and dried to give **65** (0.31 g, 73%) as a yellow solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.25−3.40 and 3.60−3.75 (m, each 4H, piperazine CH_2), 3.85 (s, 3H, NCH_3), 4.00 and 4.05 (s, each 3H, OCH_3), 6.60−6.70 and 6.75−690 (m, each 1H, pyridine CH), 7.15 and 7.25 (s, each 1H, H-1 and H-4), 7.50−7.60 (m, 1H, pyridine CH), 7.70 (s, 1H, H-5), 8.20−8.25 (m, 1H, pyridine CH), 8.75 (s, 1H, H-8).

General Procedure for Reduction of NO_2 Group. Method A. 2-Amino-6,7-dimethoxy-10-methyl-3-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (9). A solution of $SnCl_2 \cdot 2H_2O$ (0.35 g, 1.56 mmol) in 8 N HCl (15 mL) was added, at room temperature with stirring, to a solution of nitro derivative **65** (0.25 g, 0.52 mmol) in 8 N HCl (10 mL). The mixture was heated under reflux for 1 h and after cooling it was made basic to pH ∼9 with 10% NaOH solution to obtain a solid which was collected, washed several times with water, dried, purified by flash column chromatography, eluting with $CHCl_3$/MeOH (98:2), and then recrystallized by DMF to give derivative **9** (0.07 g, 33%) as a yellow solid: mp 291−293 °C; ^{1}H NMR (DMSO-d_6) δ 2.95−3.12 and 3.57−3.68 (m, each 4H, piperazine CH_2), 3.74 (s, 3H, NCH_3), 3.80 and 3.89 (s, each 3H, OCH_3), 4.82 (s, 2H, NH_2), 6.58 (dd, J = 4.91 and 7.90 Hz, 1H, pyridine CH), 6.80 (d, J = 8.6 Hz, 1H, pyridine CH), 7.00 and 7.08 (s, each 1H, H-1 and H-4), 7.40−7.58 (m, 3H, H-5, H-8, and pyridine CH), 8.08 (dd, J = 1.4 and 4.6 Hz, 1H, pyridine CH). Anal. ($C_{25}H_{27}N_5O_3$) C, H, N.

7-Fluoro-1,3-dimethoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (33). The title compound was prepared following the general Ullman (replacing 2,4-dichloro-5-nitrobenzoic acid with 2-chloro-4,5-difluorobenzoic acid), cyclization, alkylation, and coupling (120 °C, 120 h) procedures. After crystallization by EtOH, **33** was obtained in 8% overall yield as a gray solid: mp 118−119 °C; ^{1}H NMR (DMSO-d_6) δ 3.30−3.40 and 3.70−3.80 (m, each 4H, piperazine CH_2), 3.65 (s, 3H, NCH_3), 3.90 and 3.95 (s, each 3H, OCH_3), 6.25 and 6.30 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 6.65−6.85 (m, 3H, H-5 and pyridine CH), 7.55 (ddd, J = 1.8, 8.5, and 10.3 Hz, 1H, pyridine CH), 8.10 (d, J = 13.3 Hz, 1H, H-8), 8.22 (dd, J = 1.8 and 5.0 Hz, 1H, pyridine CH). Anal. ($C_{25}H_{25}FN_4O_3$) C, H, N.

1-Hydroxy-3-methoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (69).[4] A mixture of **68**[4] (0.46 g, 0.99 mmol) and LiCl (0.20 g, 4.80 mmol) in DMF (15 mL) was refluxed with stirring for 10 h. The solvent was then concentrated under reduced pressure, and the residue was poured into ice−water, made acidic (pH 6) with 2 N HCl, and extracted with $CHCl_3$. The solvent was evaporated to dryness and the crude product was purified by flash chromatography, eluting with $CHCl_3$/MeOH (98:2) to give **69** (0.30 g, 68%) as an orange solid. Melting point and spectral data are in agreement with those previously reported.[4]

General Procedure for O-Alkylation Reaction. 1-Ethoxy-3-methoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (70). A mixture of **69**[4] (0.17 g, 0.37 mmol), Cs_2CO_3 (1.20 g, 3.70 mmol), and 1-iodoethane (0.09 mL, 1.10 mmol) in dry DMF (20 mL) was heated at 90 °C for 5 h. The solvent was concentrated under reduced pressure, and the residue was poured into ice−water. The precipitate obtained was filtered, dried, and purified by flash chromatography, eluting with benzene/acetone (80:20) to give **70** (0.10 g, 55%) as a yellow solid: mp >300 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.62 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.30−3.37 (m, 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 3.76−3.84 (m, 4H, piperazine CH_2), 3.96 (s, 3H, OCH_3), 4.18 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.33 and 6.40 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 6.65−6.75 (m, 3H, H-5 and pyridine CH), 7.55 (ddd, J = 2.0, 8.1, and 9.1 Hz, 1H, pyridine CH), 8.25 (dd, J = 1.9 and 5.7 Hz, 1H, pyridine CH), 9.00 (s, 1H, H-8).

General Procedure for Reduction of NO_2 Group. Method B. 7-Amino-1-ethoxy-3-methoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (12). A stirred solution of **70** (0.10 g, 0.204 mmol) in DMF (15 mL) was hydrogenated over a catalytic amount of Raney nickel at room temperature and atmospheric pressure for 1 h. The mixture was then filtered over Celite, and the filtrate was evaporated to dryness to give a residue which was crystallized by EtOH/EtOAc to afford **12** (0.046 g, 49%) as a yellow solid: mp 215−216 °C; ^{1}H NMR (DMSO-d_6) δ 1.40 (t, J = 6.7 Hz, 3H, OCH_2CH_3), 3.09−3.20 (m, 4H, piperazine CH_2), 3.70−3.82 (m, 7H, piperazine CH_2 and NCH_3), 3.92 (s, 3H, OCH_3), 4.09 (q, J = 6.8 Hz, 2H, OCH_2CH_3), 6.30 and 6.57 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 6.70 (m, 1H, pyridine CH), 6.95 (d, J = 8.1 Hz, 1H, pyridine CH), 7.09 (s, 1H, H-5), 7.54 (s, 1H, H-8), 7.58−7.67 (m, 1H, pyridine CH), 8.12−8.18 (m, 1H, pyridine CH). Anal. ($C_{26}H_{29}N_5O_3$) C, H, N.

7-Amino-1,3-dimethoxy-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (16). The title compound was prepared from **74**[12] according to the general procedure for coupling reaction followed by reduction (method A). After flash chromatography purification, eluting with $CHCl_3$/MeOH (97:3), **16** was obtained in 35% overall yield as a yellow solid: mp 196−197 °C; ^{1}H NMR (DMSO-d_6) δ 3.05−3.15 and 3.65−3.75 (m, each 4H, piperazine CH_2), 3.85 and 3.90 (s, each 3H, OCH_3), 5.80 (bs, 2H, NH_2), 6.15 and 6.35 (bs, each 1H, H-2 and H-4), 6.60−6.80 (m, 1H, pyridine CH), 6.85−7.00 (m, 2H, H-5 and pyridine CH), 7.45 (s, 1H, H-8), 7.50−7.70 (m, 1H, pyridine CH), 8.15−8.25 (m, 1H, pyridine CH), 11.00 (s, 1H, NH). Anal. ($C_{24}H_{25}N_3O_3$) C, H, N.

7-Amino-10-ethyl-1,3-dimethoxy-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (17). The title compound was prepared from **76**[12] according to the general procedure for coupling reaction followed by reduction (method B, using DMF/2-methoxyethanol

(1:3) as solvent). After flash chromatography purification, eluting with $CHCl_3$/MeOH (97:3), **17** was obtained in 14% overall yield as a yellowish solid: mp 250−252 °C; ^{1}H NMR (DMSO-d_6) δ 1.25−1.45 (m, 3H, CH_2CH_3), 3.00−3.20 and 3.60−3.70 (m, each 4H, piperazine CH_2), 3.70 and 3.80 (s, each 3H, OCH_3), 4.20−4.40 (m, 2H, CH_2CH_3), 4.85 (bs, 2H, NH_2), 6.20 and 6.40 (bs, each 1H, H-2 and H-4), 6.55−6.60 (m, 1H, pyridine CH), 6.80−6.90 (m, 1H, pyridine CH), 7.00 (s, 1H, H-5), 7.40−7.60 (m, 2H, pyridine CH and H-8), 8.10−8.20 (m, 1H, pyridine CH). Anal. ($C_{26}H_{29}N_5O_3$) C, H, N.

7-Amino-10-isopentyl-1,3-dimethoxy-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (18). The title compound was prepared from **77** according to the general procedure for coupling reaction followed by reduction (method A). After flash chromatography purification, eluting with $CHCl_3$/MeOH (98:2), followed by crystallization by EtOH, **18** was obtained in 20% overall yield as a yellow solid: mp 246−248 °C; ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 3.4 Hz, 6H, $CH(CH_3)_2$), 1.75−2.00 (m, 3H, $NCH_2CH_2CH(CH_3)_2$), 3.10−3.25 and 3.60−3.80 (m, each 4H, piperazine CH_2), 3.90 and 4.00 (s, each 3H, OCH_3), 4.15−4.25 (m, 2H, NCH_2CH_2), 6.26 and 6.40 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 6.60−6.75 (m, 2H, pyridine CH), 6.90 (s, 1H, H-5), 7.50−7.60 (m, 1H, pyridine CH), 7.90 (s, 1H, H-8), 8.23−8.27 (m, 1H, pyridine CH). Anal. ($C_{29}H_{35}N_5O_3$) C, H, N.

7-Amino-1,3-dimethoxy-10-methyl-6-[4-(4-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (19). The title compound was prepared from **75**[12] according to the general procedure for coupling reaction employing the 1-(4-pyridinyl)piperazine followed by reduction (method A). After flash chromatography purification, eluting with $CHCl_3$/MeOH (97:3), **19** was obtained in 18% overall yield as a yellow solid: mp 297−300 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.10−3.20 and 3.50−3.60 (m, each 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 3.80 and 3.95 (s, each 3H, OCH_3), 4.90 (bs, 2H, NH_2), 6.25 and 6.50 (d, J = 1.6 Hz, each 1H, H-2 and H-4), 6.80−6.90 (m, 2H, pyridine CH), 7.00 (s, 1H, H-5), 7.50 (s, 1H, H-8), 8.10−8.20 (m, 2H, pyridine CH). Anal. ($C_{25}H_{27}N_5O_3$) C, H, N.

7-Amino-1,3,6-trimethoxy-10-methyl-9(10*H*)-acridinone (26). A mixture of **75**[12] (0.70 g, 2.0 mmol) and freshly prepared MeONa (0.34 g, 6.0 mmol) in dry DMF (40 mL) was stirred at room temperature for 40 min. After water addition (1.0 mL), the solvent was concentrated under reduced pressure to give a solid which was filtered, dried, and crystallized by DMF to give 1,3,6-trimethoxy-10-methyl-7-nitro-9(10*H*)-acridinone (0.45 g, 63%) as a yellow solid: mp 275−277 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 1H, NCH_3), 3.85, 3.90, and 4.00 (s, each 3H, OCH_3), 6.45 and 6.65 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 7.10 (s, 1H, H-5), 8.65 (s, 1H, H-8). The nitro intermediate so obtained was then reduced employing the general procedure (method A) to give after flash chromatography purification ($CHCl_3$/MeOH, 97:3) the title compound **26** in 25% yield as a brown solid: mp 295−297 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, NCH_3), 3.79, 3.87, and 3.90 (s, each 3H, OCH_3), 4.85 (bs, 2H, NH_2), 6.30 and 6.57 (bs, each 1H, H-2 and H-4), 6.95 (s, 1H, H-5), 7.43 (s, 1H, H-8). Anal. ($C_{17}H_{18}N_2O_4$) C, H, N.

7-Amino-6-chloro-1,3-dimethoxy-9(10*H*)-acridinone (40). The title compound was prepared from **74**[12] according to the general reduction procedure (method A, 2 h). After purification by flash chromatography, eluting with gradient of $CHCl_3$/MeOH (100:0 to 95:5), the title compound was obtained in 35% yield as a brown solid: mp 290−293 °C; ^{1}H NMR (DMSO-d_6) δ 3.60 and 3.70 (s, each 3H, OCH_3), 6.10 and 6.25 (bs, each 1H, H-2 and H-4), 7.20 (s, 1H, H-5), 7.40 (s, 1H, H-8), 10.90 (s, 1H, NH). Anal. ($C_{15}H_{13}ClN_2O_3$) C, H, N.

General Procedure for De-O-methylation. 7-Amino-1-hydroxy-3-methoxy-10-methyl-6-(4-phenyl-1-piperazinyl)-9(10*H*)-acridinone (27) and 7-Amino-1,3-dihydroxy-10-methyl-6-(4-phenyl-1-piperazinyl)-9(10*H*)-acridinone (30). A mixture of **22** (0.10 g, 0.22 mmol) in 48% HBr (1.5 mL) and a few drops AcOH was heated at reflux until starting material disappeared and two products formed (8 h). After the mixture was cooled, the bromohydrate precipitate was collected by filtration and solubilized in water and the solution made basic (pH 8) with saturated Na_2CO_3 solution. The precipitate so obtained was filtered and dried, and after purification by flash chromatography ($CHCl_3$/MeOH, 98:2), compound **27** was obtained in 31% yield, followed by compound **30** in 11% yield.

Compound **27**: mp 287−289 °C; ^{1}H NMR (DMSO-d_6) δ 3.10−3.20 and 3.30−3.40 (m, each 4H, piperazine CH_2), 3.60 (s, 3H, NCH_3), 3.70 (s, 3H, OCH_3), 5.00 (bs, 2H, NH_2), 6.10 and 6.40 (d, J = 2.1 Hz, 1H, H-2 and H-4), 6.80−6.85 (m, 1H, aromatic CH), 6.90−7.05 (m, 2H, aromatic CH), 7.15−7.30 (m, 3H, H-5 and aromatic CH), 7.50 (s, 1H, H-8), 15.25 (s, 1H, OH). Anal. ($C_{25}H_{26}N_4O_3$) C, H, N.

Compound **30**: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.20−3.25 and 3.30−3.35 (m, each 4H, piperazine CH_2), 4.70 (s, 3H, NCH_3), 6.00 and 6.30 (d, J = 1.8 Hz, each 1H, H-2 and H-4), 6.75−6.80 (m, 1H, aromatic CH), 6.90−7.10 (m, 2H, aromatic CH), 7.20 (s, 1H, H-5), 7.20−7.25 (m, 2H, aromatic CH), 7.50 (s, 1H, H-8), 10.40 and 14.50 (s, each 1H, OH). Anal. ($C_{24}H_{24}N_4O_3$) C, H, N.

6-Chloro-10-isopentyl-1,3-dimethoxy-7-nitro-9(10*H*)-acridone (77). The title compound was prepared from **74**[12] employing 1-iodo-3-methylbutane according to the general procedure for N-alkylation. After purification by column chromatography ($CHCl_3$/MeOH, 97:3), the title compound was obtained as a yellow solid in 24% yield: mp 209−211 °C; ^{1}H NMR (DMSO-d_6) δ 1.05 (d, J = 4.3 Hz, 6H, $CH(CH_3)_2$), 1.50−1.70 (m, 2H, NCH_2CH_2), 1.80−1.95 (m, 1H, NCH_2CH_2CH), 3.85 and 3.95 (s, each 3H, OCH_3), 4.25−4.45 (m, 2H, NCH_2CH_2), 6.50 and 6.55 (bs, each 1H, H-2 and H-4), 7.85 (s, 1H, H-5), 8.75 (s, 1H, H-8).

7-Amino-6-chloro-10-isopentyl-1,3-dimethoxy-9(10*H*)-acridinone (39). The title compound was obtained from nitro derivative **77** according to general reduction procedure (method A). After crystallization by DMF, the title compound was obtained as a yellow solid in 74% yield: mp 299−300 °C; ^{1}H NMR ($CDCl_3$) δ 1.15 (d, J = 4.3 Hz, 6H, $CH(CH_3)_2$), 1.60−1.90 (m, 3H, NCH_2CH_2CH), 3.90 and 4.00 (s, each 3H, OCH_3), 4.10−4.25 (m, 2H, NCH_2CH_2), 6.25 and 6.35 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 7.30 (s, 1H, H-5), 7.90 (s, 1H, H-8). Anal. ($C_{20}H_{23}ClN_2O_3$) C, H, N.

7-(Dimethylamino)-1,3-dimethoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (34). A mixture of **2**[4] (0.23 g, 0.52 mmol), MeI (0.3 mL, 5.2 mmol), and K_2CO_3 (0.21 g, 1.55 mmol) in dry DMF (20 mL) was heated at 90 °C for 15 h. After cooling, the reaction mixture was poured into ice−water and the yellowish precipitate was collected by filtration, dried, and purified by column chromatography ($CHCl_3$/MeOH, 98:2) followed by crystallization with Et_2O/EtOH to give **34** (0.08 g, 35%): mp 205−206 °C; ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 6H, $N(CH_3)_2$), 3.35−3.50 (m, 4H, piperazine CH_2), 3.60−3.75 (m, 7H, NCH_3 and piperazine CH_2), 3.85 and 3.90 (s, each 3H, OCH_3), 6.25 and 6.35 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 6.65−6.75 (m, 3H, pyridine CH and H-5), 7.55 (ddd, J = 2.0, 7.2, and 8.9 Hz, 1H, pyridine CH), 8.05 (s, 1H, H-8), 8.20 (dd, J = 1.9 and 5.0 Hz, 1H, pyridine CH). Anal. ($C_{27}H_{31}N_5O_3$) C, H, N.

1,3-Dimethoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (35). A solution of $NaNO_2$ (0.04 g, 0.6 mmol) in water (2 mL) was added portionwise to a stirred solution of **2**[4] (0.23 g, 0.5 mmol) in 6 N HCl, cooled at 0 °C. After 30 min, a solution of $NaBF_4$ (0.10 g, 0.9 mmol) in water (3 mL) was added and the reaction mixture was kept at 0 °C for 30 min. The borine diazonium salt formation was detected by using the β-naphthol assay. The 6,8-dimethoxy-10-methyl-9-oxo-3-[4-(2-pyridinyl)-1-piperazinyl]-9,10-dihydro-2-acridinediazonium tetrafluoroborate red precipitate was filtered and washed with a few drops of water and successively with Et_2O. The crude product, without further purification, was suspended in 50% H_3PO_2 (4 mL), and the mixture was stirred for 4 h, then poured into ice−water, made basic (pH 8) with 10% NaOH, and extracted several times with $CHCl_3$. The organic layers were then evaporated to dryness, giving a residue which was purified by flash chromatography ($CHCl_3$/MeOH, 97:3) followed by trituration with EtOAc to give **35** (0.11 g, 50%) as a white solid: mp 205−207 °C; ^{1}H NMR ($CDCl_3$) δ 3.40−3.60 (m, 4H, piperazine CH_2), 3.70−3.85 (m, 7H, piperazine CH_2, and NCH_3), 3.90 and 4.10 (s, each 3H, OCH_3), 6.25 and 6.30 (bs, each 1H, H-2 and

H-4), 6.55−6.65 (m, 3H, pyridine CH, H-5, and H-7), 6.85 (d, J = 9.0 Hz, 1H, pyridine CH), 7.50−7.60 and 8.15−8.20 (m, each 1H, pyridine CH), 8.30 (d, J = 8.9 Hz, 1H, H-8). Anal. ($C_{25}H_{26}N_4O_3$) C, H, N.

Methyl 4-Chloro-2-[*N*-(3,5-dimethoxyphenyl)-*N*-methylamino]-5-nitrobenzoate (79). The title compound was prepared starting from **78**[12] and using MeI, according to the general procedure for N-alkylation (24 h). After purification by flash chromatography (cycloexane/EtOAc, 70:30) followed by treatment with Et_2O/EtOH, the title compound was obtained in 20% yield as an orange solid: mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 3H, NCH_3), 3.50 (s, 3H, OCH_3), 3.75 (bs, 6H, OCH_3 and CH_3), 6.15−6.20 (d, J = 2.1 Hz, 2H, H-2′ and H-6′), 6.20 (t, J = 2.1 Hz, 1H, H-4′), 7.25 (s, 1H, H-3), 8.30 (s,1H, H-6).

Methyl 2-[*N*-(3,5-Dimethoxyphenyl)-*N*-methylamino]-5-nitro-4-[4-(2-pyridinyl)-1-piperazinyl]benzoate (80). The title compound was obtained starting from **79** according to the general procedure for coupling reaction. The reaction mixture was cooled obtaining a precipitate which was filtered to give **80** as an orange solid in 79% yield: mp 145−147 °C; ^{1}H NMR ($CDCl_3$) δ 3.23−3.32 (m, 4H, piperazine CH_2), 3.35 and 3.58 (s, each 3H, NCH_3 and OCH_3), 3.72 (bs, 6H, OCH_3 and CH_3), 3.75−3.82 (m, 4H, piperazine CH_2), 6.00−6.10 (m, 3H, H-2′, H-4′, and H-6′), 6.63−6.75 (m, 3H, pyridine CH and H-3), 7.58 (dt, J = 1.9 and 7.0 Hz, 1H, pyridine CH), 8.30 (dd, J = 1.9 and 5.4 Hz, 1H, pyridine CH), 8.41 (s, 1H, H-6).

Methyl 5-Amino-2-[*N*-(3,5-dimethoxyphenyl)-*N*-methylamino]-4-[4-(2-pyridinyl)-1-piperazinyl]benzoate (81). The title compound was obtained starting from **80** following the general reduction procedure (method B). After crystallization by EtOH, the title compound was obtained as a white solid in 45% yield: mp 136−138 °C; ^{1}H NMR (methanol-d_4) δ 3.00−3.10 (m, 4H, piperazine CH_2), 3.56 (s, 3H, NCH_3), 3.63−3.70 (m, 13H, OCH_3, CH_3 and piperazine CH_2), 4.75 (s, 2H, NH_2), 5.63 (d, J = 2.1 Hz, 2H, H-2′ and H-6′), 5.78 (t, J = 2.1 Hz, 1H, H-4′), 6.63−6.75 (m, 1H, pyridine CH), 6.84−6.90 (m, 2H, H-3 and pyridine CH), 7.26 (s, 1H, H-6), 7.53−7.63 (m, 1H, pyridine CH), 8.10−8.20 (m, 1H, pyridine CH).

5-Amino-2-[*N*-(3,5-dimethoxyphenyl)-*N*-methylamino]-4-[4-(2-pyridinyl)-1-piperazinyl]benzoic Acid (43). $LiOH \cdot 2H_2O$ (0.02 g, 0.54 mmol) was added to a solution of compound **81** (0.13 g, 0.27 mmol) in a dioxane/H_2O (3:1) mixture. The reaction mixture was stirred at room temperature for 2 days and then poured into water and acidified with AcOH until a precipitate formed. The solid was filtered, dried, and purified by flash chromatography ($CHCl_3$/MeOH, 99:1), giving **43** (0.012 g, 11%) as a white solid: mp 136−138 °C; ^{1}H NMR ($CDCl_3$) 2.95−3.05 (m, 4H, piperazine CH_2), 3.20 (s, 3H, NCH_3), 3.63−3.75 (m, 10H, piperazine CH_2 and OCH_3), 4.76 (bs, 2H, NH_2), 6.00−6.10 (m, 2H, H-2′ and H-6′), 6.20−6.30 (m, 1H, H-4′), 6.63−6.75 (m, 3H, H-3 and pyridine CH), 7.48−7.60 (m, 1H, pyridine CH), 7.65 (s, 1H, H-6), 8.23−8.25 (m, 1H, pyridine CH). Anal. ($C_{25}H_{29}N_5O_4$) C, H, N.

1-(5-Fluoro-2-nitrophenyl)-4-(2-pyridinyl)piperazine (82). A solution of 2,4-difluoronitrobenzene (2.1 mL, 18.0 mmol), dry Et_3N (4.5 mL, 32.0 mmol), and 1-(2-pyridinyl)piperazine (4.8 mL, 31.5 mmol) in dry toluene (10 mL) was stirred at room temperature for 2.5 h. The obtained precipitate was filtered, dried, and purified by flash chromatography (cyclohexane/EtOAc, 60:40) to give **82** (3.73 g, 58%) as an orange solid: mp 111−112 °C; ^{1}H NMR ($CDCl_3$) δ 3.10−3.20 and 3.65−3.80 (m, each 4H, piperazine CH_2), 6.60−6.85 (m, 4H, H-6, H-4, and pyridine CH), 7.45−7.60 (m, 1H, pyridine CH), 7.85 (dd, J = 6.0 and 9.0 Hz, 1H, H-3), 8.15 (ddd, J = 0.8, 2.0, and 5.0 Hz, 1H, pyridine CH). The qualitative analysis of NOESY spectra showed one relevant NOE cross-peak: piperazine CH_2/H-6.

***N*-(3,5-Dimethoxyphenyl)-*N*-methyl-4-nitro-3-[4-(2-pyridinyl)-1-piperazinyl]aniline (83).** *t*-BuOK (1.12 g, 10.0 mmol) was added portionwise to a stirred solution of *N*-methyl-3,5-dimethoxyaniline[13] (0.83 g, 5.0 mmol) in dry DMSO (15 mL) cooled at 0 °C. A solution of compound **82** (1.50 g, 5.00 mmol) in dry DMSO (8 mL) was added dropwise to this mixture. After 5 min the mixture was poured into ice−water and the solid obtained was filtered, dried, and purified by flash chromatography (cyclohexane/EtOAc, 70:30) to give **83** (0.50 g, 56%) as a yellowish solid: mp 152−154 °C; ^{1}H NMR ($CDCl_3$) δ 3.10−3.20 (m, 4H, piperazine CH_2), 3.35 (s, 3H, NCH_3), 3.70−3.75 (m, 4H, piperazine CH_2), 3.80 (bs, 6H, OCH_3), 6.25−6.40 (m, 5H, pyridine CH, H-2′, H-6′, H-2, and H-6), 6.55−6.70 (m, 2H, pyridine CH and H-4′), 7.50 (ddd, J = 2.0, 7.1, and 8.7 Hz, 1H, pyridine CH), 8.00 (d, J = 9.0 Hz, 1H, H-5), 8.15 (ddd, J = 0.7, 2.0, and 5.0 Hz, 1H, pyridine CH).

***N*^{4}-(3,5-Dimethoxyphenyl)-*N*^{4}-methyl-2-[4-(2-pyridinyl)-1-piperazinyl]-1,4-benzenediamine (44).** The title compound was prepared starting from **83** according to the general reduction procedure (method B). After purification by column chromatography (cyclohexane/EtOAc, 60:40), **44** was obtained in 82% yield as a pale-yellow solid: mp 126−128 °C; ^{1}H NMR ($CDCl_3$) δ 2.90−310 (m, 4H, piperazine CH_2), 3.20 (s, 3H, NCH_3), 3.65−3.75 (m, 10H, OCH_3 and piperazine CH_2), 4.00 (bs, 2H, NH_2), 5.80−5.90 (m, 3H, H-2′, H-6′, and H-3), 6.55−6.80 (m, 5H, H-4′, H-5, H-6, and pyridine CH), 7.50−7.60 and 8.25−8.30 (m, each 1H, pyridine CH). Anal. ($C_{24}H_{29}N_5O_2$) C, H, N.

(4-Chloro-3-nitrophenyl)(2-hydroxy-4-methoxyphenyl)methanone (84). A mixture of 1,3-dimethoxybenzene (0.25 g, 1.80 mmol), 4-chloro-3-nitrobenzoyl chloride (0.30 g, 1.48 mmol), and $AlCl_3$ (0.29 g, 1.80 mmol) in an open tube was irradiated employing microwaves (domestic microwave oven Daewoo KOR-6377) for 7 min at 57% potency. The mixture was then poured into ice−water, and the solid obtained was filtered, washed with water, then with Et_2O, and dried to give **84** (0.45 g, 65%) as a yellow solid: mp 144−145 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, OCH_3), 6.45 (dd, 1H, J = 2.3 and 9.0 Hz, H-5′), 6.55 (d, J = 2.3 Hz, 1H, H-3′), 7.40 (d, J = 9.0 Hz, 1H, H-6′), 7.65 (d, J = 8.2 Hz, 1H, H-5), 7.80 (dd, J = 1.9 and 8.2 Hz, 1H, H-6), 8.15 (d, J = 1.9 Hz, H-2), 12.20 (bs, 1H, OH). GC−MS: m/z = 307 (42) [M^+], 277 (20), 261 (14), 242 (6), 207 (7), 151 (100). The qualitative analysis of NOESY spectra showed two relevant NOE cross-peaks: 4-OCH_3/H-3; 4-OCH_3/H-5.

(2-Hydroxy-4-methoxyphenyl){3-nitro-4-[4-(2-pyridinyl)-1-piperazinyl]phenyl}methanone (85). The title compound was prepared starting from **84** by using the general procedure for the coupling reaction (70 °C for 2 h). The solvent was concentrated under reduced pressure and the orange oil was triturated with EtOH, giving a solid which was filtered and dried to give **85** (0.55 g, 97%): mp 145−147 °C; ^{1}H NMR ($CDCl_3$) δ 3.25−3.40 and 3.65−3.80 (m, each 4H, piperazine CH_2), 3.90 (s, 3H, OCH_3), 6.45 (dd, J = 2.4 and 8.8 Hz, 1H, H-5′), 6.53 (d, J = 2.4 Hz, 1H, H-3′), 6.60−6.70 (m, 2H, pyridine CH), 7.20 (d, J = 8.8 Hz, 1H, H-6′), 7.45−7.55 (m, 2H, H-5 and pyridine CH), 7.80 (dd, J = 2.0 and 8.7 Hz, 1H, H-6), 8.20−8.30 (m, 2H, H-2 and pyridine CH), 12.50 (bs, 1H, OH).

{3-Amino-4-[4-(2-pyridinyl)-1-piperazinyl]phenyl}(2,4-dimethoxyphenyl)methanone (45). The title compound was prepared starting from **85** and using MeI according to the general procedure for O-alkylation to give intermediate **86** which was reduced by the general reduction procedure (method B). After crystallization by EtOH/Et_2O, compound **45** was obtained in 57% overall yield as a yellow crystalline solid: mp 141−143 °C; ^{1}H NMR ($CDCl_3$) δ 3.00−3.20 (m, 4H, piperazine CH_2), 3.60−3.75 (m, 7H, OCH_3 and piperazine CH_2), 3.80 (s, 3H, OCH_3), 4.10 (bs, 2H, NH_2), 6.50−6.60 (m, 2H, H-3′ and H-5′), 6.65−6.75 (m, 2H, pyridine CH), 6.95 (d, J = 8.2 Hz, 1H, H-6′), 7.20 (dd, J = 2.0 and 8.2 Hz, 1H, H-6), 7.25−7.35 (m, 2H, H-5 and H-2), 7.45−7.55 (m, 1H, pyridine CH), 8.20 (m, 1H, pyridine CH). Anal. ($C_{24}H_{26}N_4O_3$) C, H, N.

{3-Amino-4-[4-(2-pyridinyl)-1-piperazinyl]phenyl}(2-hydroxy-4-methoxyphenyl)methanone (46). The title compound was prepared from **85** according to the general reduction procedure (method B). After crystallization by Et_2O/EtOH compound **46** was obtained in 60% yield as a yellow crystalline solid: mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 3.00−3.20 and 3.55−3.65 (m, each 4H, piperazine CH_2), 3.80 (s, 3H, OCH_3), 4.20 (bs, 2H, NH_2), 6.40 (dd, J = 2.51 and 8.76 Hz, 1H, H-5′), 6.50 (d, J = 2.51 Hz, 1H, H-3′), 6.65−6.80 (m, 2H, pyridine CH), 7.00−7.10 (m, 3H, H-5, H-6′,

and pyridine CH), 7.45−7.65 (m, 2H, H-6 and H-2), 8.15−8.30 (m, 1H, pyridine CH), 12.70 (bs, 1H, OH). Anal. ($C_{23}H_{24}N_4O_3$) C, H, N.

5,7-Dimethoxy-1-methyl-2-{3-nitro-4-[4-(2-pyridinyl)-1-piperazinyl]phenyl}-4(1*H*)-quinolinone (88). The title compound was prepared starting from **87**[15] by using the general procedure for coupling reaction (5 h). The addition of water gave a solid, which was filtered, dried, and crystallized by EtOH to give **88** (0.30 g, 56%) as a yellow solid: mp 215−216 °C; ^{1}H NMR ($CDCl_3$) δ 3.25−3.35 and 3.75−3.85 (m, each 4H, piperazine CH_2), 3.55 (s, 3H, NCH_3), 3.90 and 4.00 (s, each 3H, OCH_3), 6.20 (s, 1H, H-3), 6.45 (bs, 2H, H-6 and H-8), 6.65−6.75 (m, 2H, pyridine CH), 7.20 (d, J = 8.8 Hz, 1H, H-5′), 7.45−7.60 (m, 2H, H-6′ and pyridine CH), 7.85 (d, J = 2.0 Hz, 1H, H-2′), 8.20−8.25 (m, 1H, pyridine CH).

2-{3-Amino-4-[4-(2-pyridinyl)-1-piperazinyl]phenyl}-5,7-dimethoxy-1-methyl-4(1*H*)-quinolinone (47). The title compound was prepared from **88** according to the general reduction procedure (method B). After crystallization by EtOH, **47** was obtained in 91% yield as a white solid: mp 133−136 °C; ^{1}H NMR (DMSO-d_6) δ 2.90−3.00 and 3.65−3.75 (m, each 4H, piperazine CH_2), 3.50 (s, 3H, NCH_3), 3.80 and 3.90 (s, each 3H, OCH_3), 5.15 (s, 2H, NH_2), 5.75 (s, 1H, H-3), 6.45 (d, J = 1.6 Hz, 1H, H-6), 6.60−6.70 (m, 3H, H-8 and pyridine CH), 6.75 (d, J = 1.6 Hz, 1H, H-2′), 6.85 (d, J = 8.5 Hz, 1H, H-5′), 7.00 (d, J = 8.5 Hz, 1H, H-6′), 7.50−7.60 (m, 1H, pyridine CH), 8.15 (m, 1H, pyridine CH). Anal. ($C_{27}H_{29}N_5O_3$) C, H, N.

Anti-HCV Assay. Huh-5-2 cells were seeded at a density of 5 × 10^3 per well in a tissue culture treated white 96-well view plate (Packard, Canberra, Canada) in complete DMEM supplemented with 250 μg/mL G418. Following incubation for 24 h at 37 °C (5% CO_2) the medium was removed and 3-fold serial dilutions in complete DMEM (without G418) of the test compounds were added in a total volume of 100 μL. After 4 days of incubation at 37 °C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands); the luciferase signal was measured using a Luminoskan Ascent (Thermo, Vantaa, Finland). The 50% effective concentration (EC_{50}) was defined as the concentration of compound that reduced the luciferase signal by 50%.

Cytostatic Effect. Huh-5-2 cells were seeded at a density of 5 × 10^3 cells per well of a 96-well plate in complete DMEM with the appropriate concentrations of G418. Serial dilutions of the test compounds in complete DMEM without of G418 were added 24 h after seeding. Cells were allowed to proliferate for 3 days at 37 °C, after which the cell number was determined by means of the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium)/phenazinemethosulfate (MTS/PMS) method (Promega). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that inhibited the proliferation of exponentially growing cells by 50%.

Reversal of Cytostatic Effects by Guanosine. To exclude potential inhibition of the IMPDH, both the anti-HCV and cytostatic assays were performed in the presence of 100 μg/mL guanosine, similar to the method described by Neyts et al.[30]

HCV NS5B Assay. For details, see Supporting Information.

NS3 ATPase and Helicase Assays. Recombinant HCV NS3 cloned in the pAW3 plasmid (kindly provided by Dr. M. J. McGarvey, Department of Medicine, Imperial College School of Medicine, London) was expressed in *E. coli* and purified as described.[31]

ATPase Activity Assays. The NTPase activity was determined as previously described.[31]

Helicase Assays. The NS3h assay was performed as previously described in a 15 μL reaction volume containing 200 nM NS3, 5 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 25 nM ^{32}P-labeled partial duplex DNA substrate, and 5 mM ATP. The mixture was incubated for 20 min at 25 °C and then stopped with 10 μL of termination buffer (10% (w/v) glycerol, 0.0015% (v/v) bromophenol blue, 0.0015% (w/v) xylene cyanol FF, and 10 mM EDTA). Aliquots were analyzed on a native 8% (w/v) polyacrylamide gel. The intensities of the radioactive bands corresponding to ds18:66mer and ss18mer were quantified by densitometric scanning with PhosphoImager.

Topoisomerase II Mediated Decatenation and Relaxation. The topoisomerase II mediated decatenation and relaxation were determined as previously described.[32,33]

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Supporting Information Available: Table containing elemental analysis data for all the target compounds; experimental procedures and analytical data for intermediates **49**, **52**−**54**, **56**−**59**, **62**−**64**, **66**, **67**, **71**−**73** and target compounds **7**, **8**, **10**, **11**, **13**−**15**, **20**−**25**, **28**, **29**, **31**, **32**, **41** and **42**; biological protocols for HCV NS5B assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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