Supporting Information

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

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4-Chloro-2-[(3-methoxyphenyl)amino]-5-nitrobenzoic acid (49). Following the Ullman procedure of **50** and replacing the 3,4-dimethoxyaniline with 3-methoxyaniline, the title compound was obtained in 65% yield: mp 246-247 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, OCH_3), 6.80-6.90 (m, 3H, aromatic CH), 7.10 (s, 1H, H-3), 7.40 (t, *J* = 8.3 Hz, 1H, H-5'), 8.75 (s, 1H, H-6), 10.70 (bs, 1H, NH), 14.01 (bs, 1H, COOH).

4-Chloro-2-[(3,5-dimethylphenyl)amino]-5-nitrobenzoic acid (52). Following the Ullman procedure of **50** and replacing the 3,4-dimethoxyaniline with 2,3-dimethylaniline, the title compound was obtained in 51% yield: mp 290-292 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 (bs, 6H, CH_3), 6.80-7.00 (m, 4H, H-2′, H-4′, H-6 ′and H-3), 8.60 (s, 1H, H-6), 10.20 (s, 1H, NH), 14.00 (bs, 1H, COOH).

3-Chloro-2-nitro-9(10*H*)-acridinone (56). Following the cyclization procedure of **55** and starting from **51**,[1] the title compound was prepared in 65% yield: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 7.30 (t, *J* = 7.4 Hz, 1H, H-7), 7.63 (d, *J* = 7.9 Hz, 1H, H-5), 7.75 (s, 1H, H-4), 7.80-7.90 (m, 1H, H-6), 8.25 (d, *J* = 6.9, 1H, H-8), 8.80 (s, 1H, H-1), 12.5 (bs, 1H, NH).

6-Chloro-1,3-dimethyl-7-nitro-9(10*H*)-acridinone (57). Following the cyclization procedure of **55** and starting from **52**, the title compound was prepared in 30% yield: mp 298-300 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 and 2.60 (s, each 3H, CH_3), 6.70 e 6.90 (bs, each 1H, H-2 and H-4), 7.30 (s, 1H, H-5), 8.50 (s, 1H, H-8), 11.70 (bs, 1H, NH), 13,80.

3-Chloro-6-methoxy-10-methyl-2-nitro-9(10*H*)-acridinone (58) and 6-chloro-1-methoxy-10-methyl-7-nitro-9(10*H*)-acridinone (59). Following the cyclization procedure of **55** and starting from **49**, two isomers **3-chloro-6-methoxy-2-nitro-9(10*H*)-acridinone (53)** and **6-chloro-1-methoxy-7-nitro-9(10*H*)-acridinone (54),** were obtained in 70% yield, and used as a crude mixture in the next step. Following the general procedure of N-alkylation of **60**, starting from the mixture of **53** and **54**, compounds **58** and **59** were obtained after column chromatography purification eluting with a gradient of CH_2Cl_2/MeOH (100:0 to 98:2). Compound **58** was obtained in 38% yield followed by compound **59** obtained in 42% yield.
Compound **58**: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 7.05 (dd, *J* = 2.05 and 8.80 Hz, 1H, H-7), 7.20 (d, *J* = 2.0 Hz, 1H, H-5), 8.10 (s, 1H, H-4), 8.20 (d, *J* = 8.80 Hz, 1H, H-8), 8.80 (s, 1H, H-1).
Compound **59**: mp> 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 6.80 (d, *J* = 8.3 Hz, H-2), 7.25 (d, *J* = 8.5 Hz, H-4), 7.65 (t, *J* = 8.9 Hz, H-3), 8.10 (s, 1H, H-5), 8.80 (s, 1H, H-8).

6-Chloro-1,3,10-trimethyl-7-nitro-9(10*H*)-acridinone (62). Following the general procedure of N-alkylation of **60** and staring from **57**, the title compound was obtained in 75% yield: mp >300 °C; ^{1}H NMR (DMSO-d_6/TFA) δ 2.25 e 2.50 (s, each 3H, CH_3), 3.65 (s, 3H, NCH_3), 6.75-6.80 (m, 2H, H-2 and H-4), 7.35 (s, 1H, H-5), 8.60 (s, 1H, H-8).

6-Methoxy-10-methyl-2-nitro-3-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (63). Following the general procedure for coupling reaction of **65** and starting from **58**, the title compound was obtained in 60% yield: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.25-3.35 and 3.60-3.70 (m, each 4H, piperazine CH_2), 3.80 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 6.60-6.70 (m, 1H,

pyridine CH), 6.65-6.75 (m, 2H, H-5, and H-7), 7.10-7.20 (m, 2H, H-4, and pyridine CH), 7.50-7.60 (m, 1H, pyridine CH), 8.10-8.20 (m, 2H, H-8, and pyridine CH), 8.75 (s, 1H, H-1).

1-Methoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (64). Following the general procedure for coupling reaction of **65** and starting from **59,** the title compound was obtained in 36% yield: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.30 and 3.60-3.75 (m, each 4H, piperazine CH_2), 3.80 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 6.60-6.70 (m, 2H, H-3 and pyridine CH), 6.85 (d, J = 8.6 Hz, 1H, H-4), 7.10 (s, 1H, H-5), 7.20 (d, J = 8.6 Hz, 1H, H-2), 7.60-7.70 (m, 2H, pyridine CH), 8.10-8.20 (m, 1H, pyridine CH), 8.75 (s, 1H, H-8).

10-Methyl-2-nitro-3-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (66). Following the general procedure for coupling reaction of **65** and starting from **61,** the title compound was obtained in 60% yield: mp 215-216 °C; ^{1}H NMR (DMSO-d_6) δ 3.40-3.60 and 3.70-3.90 (m, each 4H, piperazine CH_2), 4.00 (s, 3H, NCH_3), 6.85 (t, J = 6.9 Hz, 1H, pyridine CH), 7.10 (d, J = 8.7 Hz, 1H, pyridine CH), 7.15 (s, 1H, H-4), 7.45 (ddd, J = 5.5, 7.6 and 9.4 Hz, 1H, H-7), 7.75 (t, J = 8.3 and 0.5 Hz, 1H, pyridine CH), 7.85-7.90 (m, 2H, H-5 and H-6), 8.20 (dd, J = 1.4 and 5.0 Hz, 1H, pyridine CH), 8.35 (d, J = 7.7 Hz, 1H, H-8), 8.80 (s, 1H, H-1).

1,3,10-trimethyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (67). Following the general procedure for coupling reaction of **65** and starting from **62,** the title compound was obtained in 80% yield: mp 250-251 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 and 2.80 (s, each 3H, CH_3), 3.30-3.45 and 3.60-3.75 (m, each 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 6.60-6.75 (m, 1H, pyridine CH), 6.80-7.10 (m, 3H, H-2, H-4 and pyridine CH), 7.40 (s, 1H, H-5), 7.50-7.70 (m, 1H, pyridine CH), 8.10-8.25 (m, 1H, pyridine CH), 8.70 (s, 1H, H-8).

2-Amino-6-methoxy-10-methyl-3-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (7). Following the reduction procedure of **9** and starting from **63**, the title compound was obtained after crystallization by EtOH/DMF, in 36% yield as yellow solid: mp 266-267 °C; ^{1}H NMR (DMSO-d_6) δ 3.10-3.20 (m, 4H, piperazine CH_2), 3.70-3.75 (m, 7H, NCH_3, and piperazine CH_2), 3.95 (s, 3H, OCH_3), 6.60-6.85 (m, 4H, H-5, H-7, and pyridine CH), 7.00 (s, 1H, H-4), 7.50-7.60 (m, 1H, pyridine CH), 7.85 (s, 1H, H-1), 8.35 (m, 1H, pyridine CH). Anal. ($C_{24}H_{25}N_5O_2$) C, H, N.

7-Amino-1-methoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (8). Following the reduction procedure of **9** and starting from **64**, the title compopund was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 97:3), in 23% yield as yellow solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.15 and 3.60-3.75 (m, each 4H, piperazine CH_2), 3.80 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 5.00 (bs, 2H, NH_2), 6.60-6.75 (m, 2H, H-2 and pyridine CH), 6.85 (d, J = 8.6 Hz, 1H, pyridine CH), 7.15 (s, 1H, H-5), 7.20 (d, J = 8.7 Hz, 1H, H-4), 7.50-7.60 (m, 3H, H-3, H-8, and pyridine CH), 8.15 (m, 1H, pyridine CH). Anal. ($C_{24}H_{25}N_5O_2$) C, H, N.

2-Amino-10-methyl-3-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (10). Following the reduction procedure of **9** and starting from **66**, the title compopund was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 97:3), in 25% yield as yellow solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.10-3.30 and 3.60-3.80 (m, each 4H, piperazine CH_2), 3.90 (s, 3H, NCH_3) 5.00 (bs, 2H, NH_2), 6.60-6.70 (m, 1H, pyridine CH), 6.85-6.95 (m, 1H, pyridine CH), 7.20-7.25 (m, 2H, H-4 and H-7), 7.50-7.70 (m, 2H, H-1 and H-6), 7.70-7.75 (m, 2H, H-5 and pyridine CH), 8.15 (dd, J = 1.9 and 5.0 Hz, 1H, pyridine CH), 8.25 (dd, J = 2.1 and 9.9 Hz, 1H, H-8). Anal. ($C_{23}H_{23}N_5O$) C, H, N.

7-Amino-1,3,10-trimethyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (11). Following the reduction procedure of **9** and starting from **67**, the title compound was obtained after

purification by flash chromatography ($CHCl_3$/MeOH, 97:3) followed by crystallization with EtOH, in 24% yield as yellow solid: mp 218-220 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 and 2.80 (s, each 3H, CH_3), 3.00-3.54 and 3.62-3.75 (m, each 4H, piperazine CH_2), 3.80 (s, 3H, NCH_3), 4.87 (bs, 2H, NH_2), 6.62-6.73 (m, 1H, pyridine CH), 6.79 (s, 1H, H-2), 6.87 (d, *J* = 8.4 Hz, 1H, pyridine CH), 7.10 (s, 1H, H-4), 7.35 (s, 1H, H-5), 7.45-7.65 (m, 2H, pyridine CH and H-8), 8.10-8.20 (m, 1H, pyridine CH). Anal. ($C_{25}H_{27}N_5O$) C, H, N.

3-Methoxy-10-methyl-7-nitro-1-propoxy-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (71). Following the general procedure for *O*-alkylation of compound **70** and replacing the 1-iodoethane with 1-iodopropane, the title compound was obtained in 91% yield: mp 284-285 °C; ^{1}H NMR (DMSO d_6) δ 1.12 (t, *J* = 7.13 Hz, 3H, $OCH_2CH_2CH_3$), 1.78 (q, *J* = 7.1 Hz, 2H, $OCH_2CH_2CH_3$), 3.25-3.40 and 3.70-3.76 (m, each 4H, piperazine CH_2), 3.80 and 3.90 (s, each 3H, NCH_3 and OCH_3), 4.02 (t, *J* = 7.1 Hz, 2H, $OCH_2CH_2CH_3$), 6.45 and 6.60 (d, *J* = 2.0 Hz, each 1H, H-2 and H-4), 6.70 (dd, *J* = 2.0 and 7.0 Hz, 1H, pyridine CH), 6.90 (d, *J* = 8.6 Hz, 1H, pyridine CH), 7.00 (s, 1H, H-5), 7.63 (ddd, *J* = 1.8, 7.0 and 8.9 Hz, 1H, pyridine CH), 8.18 (dd, *J* = 1.4 and 4.8 Hz, 1H, pyridine CH), 8.60 (s, 1H, H-8).

1-Isobutoxy-3-methoxy-10-methyl-7-nitro-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (72). Following the general procedure for *O*-alkylation of compound **70** and replacing the 1-iodoethane with 2-methyl-1-iodopropane, the title compound was obtained after flash chromatography purification ($CHCl_3$ /MeOH, 99:1), in 45% yield: mp 294-296 °C ; ^{1}H NMR ($CDCl_3$) δ 1.08 (d, *J* = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$), 2.10-2.31 (m, 1H, $OCH_2CH(CH_3)_2$), 3.15-3.27 (m, 4H, piperazine CH_2), 3.60-3.79 (m, 9H, NCH_3, piperazine CH_2 and $OCH_2CH(CH_3)_2$), 3.85 (s, 1H, OCH_3), 6.18 and 6.23 (d, *J* = 2.11 Hz, each 1H, H-2 and H-4), 6.53-6.69 (m, 3H, H-5 and pyridine CH), 7.44 (dt, *J* = 2.0 and 9.0 Hz, 1H, pyridine CH), 8.14 (dd, *J* = 2.0 and 5.2 Hz, 1H, pyridine CH), 8.91 (s, 1H, H-8).

({3-Methoxy-10-methyl-7-nitro-9-oxo-6-[4-(2-pyridinyl)-1-piperazinyl]-9,10-dihydro-1-acridinyl}oxy)acetic acid (73). Following the general procedure for *O*-alkylation of compound **70** and replacing the 1-iodoethane with ethyl α-bromoacetate, the title compound was obtained after crystallization by EtOH/DMF (2:1) in 39% yield: mp 252-253°C; ^{1}H NMR (DMSO-d_6) δ 3.10-3.40 and 3.65-3.70 (m, each 4H, piperazine CH_2), 3.75 (s, 3H, OCH_3), 3.90 (s, 3H, NCH_3), 4.90 (s, 2H, OCH_2), 6.40 (d, *J* = 2.0 Hz, 1H, H-2), 6.60-6.75 (m, 2H, H-4 and pyridine CH), 6.85-7.00 (m, 2H, H-5 and pyridine CH), 7.50-7.65 (m, 1H, pyridine CH), 8.10-8.20 (dd, *J* = 1.6 and 4.9 Hz, 1H, pyridine CH), 8.60 (s, 1H, H-8).

7-Amino-3-methoxy-10-methyl-1-propoxy-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (13). Following the reduction procedure of **12** and starting from **71**, the title compound was obtained after crystallized by EtOH/DMF, in 55% yield as yellow solid: mp 264-265 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 1.70-1.80 (m, 2H, CH_2CH_3), 3.00-3.25 (m, 4H, piperazine CH_2), 3.70-3.80 (m, 7H, piperazine CH_2 and NCH_3), 3.82-4.00 (m, 5H, OCH_3 and OCH_2), 4.80 (bs, 2H, NH_2), 6.25 and 6.55 (d, *J* = 1.60 Hz, each 1H, H-2 and H-4), 6.65 (dd, *J* = 1.9 and 7.0 Hz, 1H, pyridine CH), 6.90 (d, *J* = 8.5 Hz, 1H, pyridine CH), 7.05 (s, 1H, H-5), 7.50-7.65 (m, 2H, H-8 and pyridine CH), 8.20-8.35 (m, 1H, pyridine CH). Anal. ($C_{27}H_{31}N_5O_3$) C, H, N.

7-Amino-1-isobutoxy-3-methoxy-10-methyl-6-[4-(2-pyridinyl)-1-piperazinyl]-9(10*H*)-acridinone (14). Following the reduction procedure of **12** and starting from **72**, the title compound was obtained after crystallization by EtOH/DMF, in 32% yield as yellow solid: mp 245-247 °C; ^{1}H NMR ($CDCl_3$) δ 1.17 (d, *J* = 6.7 Hz, 6H, $CH(CH_3)_2$), 2.22-2.40 (m, 1H, $CH(CH_3)_2$), 3.17-3.23 (m, 4H, piperazine CH_2), 3.70-3.79 (m, 7H, piperazine CH_2 and NCH_3), 3.84 (d, *J* = 6.8 Hz, 2H, OCH_2),

3.93 (s, 3H, OCH_3), 6.24 and 6.35 (d, J = 2.1 Hz, each 1H, H-2 e H-4), 6.65-6.78 (m, 2H, pyridine CH), 6.93 (s, 1H, H-5), 7.55 (ddd, J = 2.0, 6.9 and 8.9 Hz, 1H, pyridine CH), 7.86 (s, 1H, H-8), 8.24 (dd, J = 1.2 and 4.9 Hz, 1H, pyridine CH). Anal. ($C_{28}H_{33}N_5O_3$) C, H, N.

({7-Amino-3-methoxy-10-methyl-9-oxo-6-[4-(2-pyridinyl)-1-piperazinyl]-9,10-dihydro-1-acridinyl}oxy)acetic acid (15). Following the reduction procedure of **12** and starting from **73**, the title compopund was obtained after purification by reverse flash chromatography (dioxane/H_2O, 99:1), in 17% yield as yellow solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.25 and 3.60-3.80 (m, each 4H, piperazine CH_2), 3.90 and 4.00 (s, each 3H, NCH_3 and OCH_3), 4.80 (s, 2H, OCH_2), 5.20 (bs, 2H, NH_2) 6.50 (bs, 1H, H-2), 6.55-6.80 (m, 2H, H-4 and pyridine CH), 6.90 (d, J = 7.8 Hz, 1H, pyridine CH), 7.20 (s, 1H, H-5), 7.50-7.70 (m, 2H, pyridine CH and H-8), 8.10-8.25 (m, 1H, pyridine CH). Anal. ($C_{26}H_{27}N_5O_5$) C, H, N.

7-Amino-1,3-dimethoxy-10-methyl-6-[4-(2-pyrimidinyl)-1-piperazinyl]-9(10*H*)-acridinone (20). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 2-(1-piperazinyl)pyrimidine, the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 93:7), in 21% overall yield as a yellow solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.10 and 3.95-4.00 (m, each 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 3.80 and 3.90 (s, each 3H, OCH_3), 4.75 (bs, 2H, NH_2), 6.26 and 6.55 (d, J = 1.8 Hz, each 1H, H-2 and H-4), 6.65 (t, J = 4.7 Hz, 1H, pyrimidine CH), 7.05 (s, 1H, H-5), 7.50 (s, 1H, H-8), 8.30-8.35 (m, 2H, pyrimidine CH). Anal. ($C_{24}H_{26}N_6O_3$) C, H, N.

7-Amino-1,3-dimethoxy-10-methyl-6-{4-[5-(trifluoromethyl)-2-pyridinyl]-1-piperazinyl}-9(10*H*)-acridinone (21). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 1-[5-(trifluoromethyl)-2-pyridinyl]piperazine the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 95:5), in 20% overall yield as a yellow solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.15 (m, 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 3.80 (s, 3H, OCH_3), 3.90-4.00 (m, 7H, piperazine CH_2 and OCH_3), 4.90 (bs, 2H, NH_2), 6.25 and 6.55 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 7.00-7.10 (m, 2H, pyridine CH, and H-5), 7.50 (s, 1H, H-8), 7.80 (dd, J = 2.5 and 9.2 Hz, 1H, pyridine CH), 8.45 (d, J = 0.9 Hz, 1H, pyridine CH). Anal. ($C_{26}H_{26}F_3N_5O_3$) C, H, N.

7-Amino-1,3-dimethoxy-10-methyl-6-(4-phenyl-1-piperazinyl)-9(10*H*)-acridinone (22). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 1-phenylpiperazine, the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 97:3), in 39% overall yield as a yellow solid: mp 275-276 °C; ^{1}H NMR (DMSO-d_6) δ 3.10-3.20 and 3.30-3.40 (m, each 4H, piperazine CH_2), 3.75 (s, 3H, NCH_3), 3.80 and 3.90 (s, each 3H, OCH_3), 4.80 (s, 2H, NH_2), 6.25 and 6.55 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 6.70-6.85 (m, 1H, aromatic CH), 6.90-7.10 (m, 3H, aromatic CH and H-5), 7.15-7.28 (m, 2H, aromatic CH), 7.40 (s, 1H, H-8). Anal. ($C_{26}H_{28}N_4O_3$) C, H, N.

7-Amino-1,3-dimethoxy-10-methyl-6-[4-(1,3-thiazol-2-yl)-1-piperazinyl]-9(10*H*)-acridinone (23). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 1-(1,3-thiazol-2-yl)piperazine, the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 93:7), in 35% overall yield as a yellow solid: mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 3.20-3.30 (m, 4H, piperazine CH_2), 3.60-3.70 (m, 7H, piperazine CH_2 and NCH_3), 3.80 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), 6.60 (d, J = 3.7 Hz, 1H, thiazole CH), 6.80 (s, 1H, H-5), 7.25 (d, J = 3.7 Hz, 1H, thiazole CH), 7.80 (s, 1H, H-8). Anal. ($C_{23}H_{25}N_5O_3S$) C, H, N.

7-Amino-6-[4-(1,3-benzoxazol-2-yl)-1-piperazinyl]-1,3-dimethoxy-10-methyl-9(10*H*)-acridinone (24). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 2-(1-piperazinyl)-1,3-benzoxazole,[2] the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 93:7), in 38% overall yield as a yellow solid: mp 284-286 °C; ^{1}H NMR (DMSO-d_6) δ 3.00-3.25 (m, 4H, piperazine CH_2), 3.65-3.80 (m, 7H, piperazine CH_2 and NCH_3), 3.90 and 4.00 (s, each 3H, OCH_3), 4.95 (bs, 2H, NH_2), 6.25 and 6.50 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 6.85-7.00 (m, 2H, H-5 and aromatic CH), 7.10 (t, J = 7.6 Hz, 1H, aromatic CH), 7.25 and 7.35 (d, J = 7.6 Hz, each 1H, aromatic CH), 7.50 (s, 1H, H-8). Anal. ($C_{27}H_{27}N_5O_4$) C, H, N.

7-Amino-1,3-dimethoxy-6-[4-(2-methoxyphenyl)-1-piperazinyl]-10-methyl-9(10*H*)-acridinone (25). Following the same procedure as that used for the synthesis of **19** and replacing 1-(4-pyridinyl)piperazine with 1-(2-methoxyphenyl)piperazine, the title compound was obtained after purification by flash chromatography ($CHCl_3$/MeOH, 98:2), in 10% overall yield as a yellow solid: mp 271-272 °C; ^{1}H NMR δ ($CDCl_3$) 3.27-3.35 (m, 8H, piperazine CH_2), 3.78, 3.92 and 3.95 (s, each 3H, OCH_3), 4.00 (s, 3H, NCH_3), 6.26 and 6.32 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 6.83-7.20 (m, 5H, H-5 and aromatic H), 7.78 (s, 1H, H-8). Anal. ($C_{27}H_{30}N_4O_4$) C, H, N.

7-Amino-6-[4-(1,3-benzoxazol-2-yl)-1-piperazinyl]-1-hydroxy-3-methoxy-10-methyl-9(10*H*)-acridinone (28) and 7-amino-6-[4-(1,3-benzoxazol-2-yl)-1-piperazinyl]-1,3-dihydroxy-10-methyl-9(10*H*)-acridinone (31). Following the de-*O*-methylation procedure of **27** and **30**, and starting from **24**, the title compounds were obtained after purification by flash chromatography ($CHCl_3$/MeOH, 97:3). Compound **28** was obtained in 41% yield, followed by compound **31** in 14% yield.

Compound **28**: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.10-3.30 and 3.85-3.90 (m, each 4H, piperazine CH_2), 3.80 and 4.05 (s, each 3H, NCH_3 and OCH_3), 5.00 (bs, 2H, NH_2), 6.30 and 6.60 (d, J = 2.0 Hz, each 1H, H-2 and H-4), 7.00-7.15 (m, 2H, aromatic CH and H-5), 7.20 (t, J = 6.2 Hz, 1H, aromatic CH), 7.35 and 7.45 (d, J = 7.2 Hz, each 1H, aromatic CH), 7.70 (s, 1H, H-8), 15.50 (s, 1H, OH). Anal. ($C_{26}H_{25}N_5O_4$) C, H, N.

Compound **31**: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 2.70-3.00 (m, each 4H, piperazine CH_2), 3.50 (s, 3H, NCH_3), 5.00 (bs, 2H, NH_2), 6.00 and 6.40 (bs, each 1H, H-2 and H-4), 7.00-7.30 (m, 3H, H-5 and aromatic CH), 7.40 and 7.50 (d, J = 7.5 Hz, each 1H, aromatic CH), 7.55 (s, 1H, H-8), 10.50 and 15.00 (s, each 1H, OH). Anal. ($C_{25}H_{23}N_5O_4$) C, H, N.

7-Amino-1-hydroxy-3,6-dimethoxy-10-methyl-9(10*H*)-acridinone (29) and 7-amino-1,3-dihydroxy-6-methoxy-10-methyl-9(10*H*)-acridinone (32). Following the de-*O*-methylation procedure of **27** and **30**, and starting from **26**, the title compounds were obtained after purification by flash chromatography ($CHCl_3$/MeOH, 95:5). Compound **29** was obtained in 37% yield, followed by compound **32** in 10% yield.

Compound **29**: mp 287-288 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, NCH_3), 3.90 and 4.00 (s, each 3H, OCH_3), 5.00 (s, 2H, NH_2), 6.00 and 6.50 (d, J = 2.1 Hz, each 1H, H-2 and H-4), 7.15 (s, 1H, H-5), 7.50 (s, 1H, H-8), 15.70 (s, 1H, OH). Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

The qualitative analysis of NOESY spectra showed three relevant NOE cross-peaks: 3-OCH_3/H-2; 3-OCH_3/H-4; 6-OCH_3/H-5.

Compound **32**: mp > 300 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 3.35 and 4.00 (s, each 3H, NCH_3 and OCH_3), 5.00 (bs, 2H, NH_2), 5.90 and 6.35 (d, J = 1.9 Hz, each 1H, H-2 and H-4), 7.00 (s, 1H, H-5), 7.45 (s, 1H, H-8), 10.10 and 15.70 (s, each 1H, OH). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

The qualitative analysis of NOESY spectra showed one relevant NOE cross-peak: 6-OCH_3/H-5.

7-Amino-6-chloro-1-hydroxy-3-methoxy-9(10*H*)-acridinone (41) and 7-Amino-6-chloro-1,3-dihydroxy-9(10*H*)-acridinone (42). Following the de-*O*-methylation procedure of **27** and **30**, and

starting from **40**, the title compounds were obtained after purification by flash chromatography (CH_2Cl_2/EtOAc/MeOH, 98:2:0 to 97.5:2:0.5). Compound **41** was obtained in 27% yield followed by **42** in 10% yield.
Compound **41**: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H, OCH_3), 5.50 (s, 2H, NH_2), 6.05 and 6.25 (d, J = 2.2 Hz, each 1H, H-2 and H-4), 7.30 (s, 1H, H-5), 7.55 (s, 1H, H-8), 11.80 (bs, 1H, NH), 14.20 (bs, 1H, OH). Anal. ($C_{14}H_{11}ClN_2O_3$) C, H, N.
Compound **42**: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 5.50 (bs, 2H, NH_2), 5.90 and 6.10 (bs, 2H, H-2 and H-4), 7.40 (s, 1H, H-5), 7.60 (s, 1H, H-8), 11.50 (bs, 2H, OH and NH), 14.20 (s, 1H, OH). Anal. ($C_{13}H_9ClN_2O_3$) C, H, N.

Biological Protocols

HCV NS5B Assays. The cloned gene for HCV NS5B was kindly provided by Dr. Joachim Jaeger, Astbury Centre, RepliZyme Ltd, UK. Recombinant HCV NS5B was expressed in *E. coli* and purified by FPLC chromatography through DEAE and HyperD Heparin columns. Assays were performed as follows: NS5B (25-50 nM) was incubated in a final volume of 25 µl in the presence of 50 mM Tris-HCl pH8.0, 1 mM DTT, 0.25 mg/ml BSA, 2.5 µM (3'-OH ends) of the RNA/RNA homopolymeric template $(rA)_{40}/(rU)_{20}$, 0.25 mM $MnCl_2$ and 2 µM [^{3}H]-UTP (37Ci/mmol). Samples were incubated 40 min at 25°C. 20 µl aliquots were spotted on DE-81 filters (Wathman) which were washed twice in 0.5 M K_2HPO_4, once in water and once in acetone. Bound radioactivity was detected by liquid scintillation counter (Microbeta Trilux, Perkin-Elmer).

References

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Elemental Analysis data for target compounds 7-35 and 39-47

compd	calcd			found		
	C%	H%	N%	C%	H%	N%
7	69.38	6.06	16.86	68.08	6.17	16.92
8	69.38	6.06	16.86	69.28	5.85	16.66
9	67.40	6.11	15.72	67.65	6.32	15.81
10	71.67	6.01	18.17	71.38	6.05	18.10
11	72.61	6.58	16.94	72.95	6.66	16.86
12	67.95	6.36	15.24	67.91	6.30	14.91
13	68.48	6.60	14.79	68.28	6.51	14.76
14	68.97	6.82	14.36	68.69	6.76	14.16
15	63.79	5.56	14.31	63.75	5.49	14.28
16	66.81	5.84	16.23	67.09	6.02	16.28
17	67.95	6.36	15.24	68.05	6.39	15.22
18	69.44	7.03	13.96	69.38	6.98	13.89
19	67.40	6.11	15.72	67.36	6.01	15.69
20	64.56	5.87	18.82	64.51	5.81	18.79
21	60.81	5.10	11.10	61.01	5.01	11.07
22	70.25	6.35	12.60	70.23	6.31	12.56
23	61.18	5.58	15.51	61.07	5.52	15.48
24	66.79	5.61	14.42	66.59	5.59	14.40
25	68.34	6.37	11.81	68.31	6.32	11.92
26	64.96	5.77	8.91	65.03	5.71	8.88
27	69.75	6.09	13.01	69.73	6.12	13.08
28	66.23	5.34	14.85	66.41	5.39	14.89
29	63.99	5.37	9.33	64.21	5.49	9.05
30	69.21	5.81	13.45	69.18	5.78	13.39
31	65.63	5.07	15.31	65.28	4.98	15.51
32	62.93	4.93	9.79	63.12	5.21	9.59
33	66.95	5.62	12.49	66.64	5.58	12.39
34	68.48	6.60	14.79	68.38	6.53	14.69
35	69.75	6.09	13.01	69.73	6.05	12.98
39	64.08	6.18	7.47	64.05	6.10	7.39
40	59.12	4.30	9.19	60.01	4.27	9.08
41	57.84	3.81	9.64	57.65	4.03	9.55
42	56.43	3.28	10.13	56.33	3.21	10.22
43	64.78	6.31	15.11	64.65	6.28	15.15
44	68.71	6.97	16.69	68.59	6.76	16.76
45	68.88	6.26	13.39	68.93	6.34	13.35
46	68.30	5.98	13.85	68.21	6.05	13.77
47	68.77	6.20	14.85	68.87	6.18	15.01