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Discovery of naturally occurring aurones that are potent allosteric inhibitors of hepatitis C virus RNA-dependent RNA polymerase

Romain Haudecoeur [1†], Abdelhakim Ahmed-Belkacem [2†], Wei Yi [1], Antoine Fortuné [1], Rozenn Brillet [2], Catherine Belle [3], Edwige Nicolle [1], Coralie Pallier [2 4], Jean-Michel Pawlotsky [2 5], Ahcène Boumendjel [1*]

[1] *DPM, Département de pharmacochimie moléculaire CNRS : UMR5063 , Université Joseph Fourier - Grenoble I , Batiment E André Rassat, 470 rue de la chimie - BP 53 - 38041 Grenoble Cedex 9, FR*

[2] *Institut Mondor de Recherche Biomédicale INSERM : U955 , Université Paris XII - Paris Est Créteil Val-de-Marne , IFR10 , 8 rue du Général Sarrail, 94010 Créteil, FR*

[3] *DCM, Département de Chimie Moléculaire CNRS : UMR5250 , Université Joseph Fourier - Grenoble I , 301, rue de la Chimie 38041 Grenoble cedex 9,FR*

[4] *Laboratoire de virologie Hôpital Bicêtre , Assistance publique - Hôpitaux de Paris (AP-HP) , 84 rue du Général Leclerc 94276 Le Kremlin-Bicêtre Cedex, FR*

[5] *Centre National de Référence Virus des hépatites B, C et Delta Institut National de la Transfusion Sanguine , Assistance publique - Hôpitaux de Paris (AP-HP) , 6 rue Alexandre Cabanel 75015 Paris, FR*

* Correspondence should be addressed to: Ahcène Boumendjel <Ahcene.Boumendjel@ujf-grenoble.fr >

† Equal contribution to this work

Equal senior investigators in this study

Abstract

We have identified naturally occurring 2-benzylidenebenzofuran-3-ones (aurones) as new templates for non-nucleoside hepatitis C virus (HCV) RNA-dependent RNA polymerase (RdRp) inhibitors. The aurone target site, identified by site-directed mutagenesis, is located in Thumb Pocket I of HCV RdRp. The RdRp inhibitory activity of 42 aurones was rationally explored in an enzyme assay. Molecular docking studies were used to determine how aurones bind to HCV RdRp and to predict their range of inhibitory activity. Seven aurone derivatives were found to have potent inhibitory effects on HCV RdRp, with IC_{50}s below 5 μM and excellent selectivity. The most active aurone analogue was (*Z*)-2-((1-butyl-1*H* -indol-3-yl)methylene)-4,6-dihydroxybenzofuran-3(2*H*)-one (compound 51), with an IC_{50} of 2.2 μM. Their potent RdRp inhibitory activity, together with their low toxicity, make these molecules attractive candidate direct-acting anti-HCV agents.

MESH Keywords Antiviral Agents ; chemical synthesis ; pharmacology ; Benzofurans ; chemical synthesis ; pharmacology ; Hepacivirus ; enzymology ; Models, Molecular ; RNA Replicase ; antagonists & inhibitors ; metabolism

Introduction

Hepatitis C virus (HCV) infection is a global public health problem. The World Health Organisation (WHO) estimates that 120–140 million people are infected worldwide (2–3% of the world population), while 3–4 million are newly infected each year. Chronic HCV infection is associated with chronic liver inflammation and fibrosis. In the absence of antiviral treatment, HCV liver disease progresses to cirrhosis in 20–30% of chronically infected patients, and patients with cirrhosis have a 1% to 4% annual risk of developing hepatocellular carcinoma.1 HCV infection is curable. The current standard of care is a combination of pegylated interferon alpha (peg-IFN) and ribavirin (RBV).2 –5 Unfortunately, the response rate is low, especially among patients infected by HCV genotype 1, the most frequent genotype in the U.S., Europe, Asia, and Latin America (60 to 75% of cases).6 In 2012, a new treatment based on a combination of peg-IFN, ribavirin and a direct-acting antiviral drug that inhibits HCV serine protease activity (telaprevir or boceprevir) will be available for patients with HCV genotype 1 infection. Yet these new therapies will fail in 20–30% of treatment-naïve patients and in about 50% of patients in whom a first course of peg-IFN and ribavirin has failed to eradicate the infection. Thus, as a preventive vaccine will not be available for several years, there is an urgent need for more effective anti-HCV drugs.

The HCV RNA-dependent RNA polymerase (RdRp) is a particularly attractive target because of its key role in viral replication and the fact that this enzyme has no functional equivalent in mammalian cells, thus ensuring its selectivity. A number of HCV RdRp inhibitors have been identified, including nucleoside/nucleotide analogues that target the active site, and non-nucleoside allosteric inhibitors.7 ,8 The HCV RdRp structure resembles a right hand. Four allosteric binding sites have been identified so far, including “Thumb” Pockets I and II and “Palm” Pockets I and II.8 ,9 To date, two classes of molecules sharing a number of similarities have been reported to bind to Thumb Pocket I, including 2-aryl-1-cyclohexylbenzimidazole-5-carboxylic acid derivatives and their indole-based equivalents (Figure 1).10 –16

Some of these compounds are currently being tested in HCV-infected patients. For example, a phase I clinical trial of MK-3281, an indole-based Thumb Pocket I inhibitor, has recently been completed,17 but this drug will not be further developed because of safety concerns.

Aurones (2-benzylidenebenzofuran-3-ones) are naturally occurring small molecules belonging to the flavonoid family, the pharmacological potential of which we reported for the first time several years ago.18 As part of an ongoing program investigating the therapeutic potential of aurones, we discovered that aureusidin (3′,4,4′,6-tetrahydroxyaurone, **1** , Figure 1),19 ,20 a naturally occurring aurone, potently inhibits HCV RdRp, with an IC_{50} of 5.2 μM and a selectivity index of 90. Mutagenic studies and molecular modeling identified Thumb Pocket I as the aurone binding site. Based on these preliminary data, we used the aurone scaffold as a template for the design of aurone analogues with potent inhibitory activity on HCV RdRp.

Chemistry

The aurone derivatives described in this study were synthesized by means of aldol condensation of a substituted benzofuran-3(2*H*)-one with a benzaldehyde derivative, in basic KOH/MeOH conditions under reflux. Aurones bearing fluoro groups were obtained with a softer method using neutral alumina, as described by Varma *et al.* , in order to avoid nucleophilic substitutions by *in situ* -formed potassium methoxide.21 4,6-dihydroxyaurones were synthesized by preparing the corresponding 4,6-dimethoxy analogues, followed by deprotection with boron tribromide. In some cases it was possible to obtain these derivatives directly by simple basic condensation in KOH/EtOH (Scheme 1).

These pathways required the synthesis of benzofuran-3(2*H*)-ones **2** to **9** by various methods, as described elsewhere (Scheme 2). Compounds **2** and **3** were synthesized from resorcinol and phloroglucinol, respectively. Using chloroacetonitrile in HCl/Et_2O, electrophilic addition in *ortho* position from one of the hydroxyl groups allowed the recovery of the chloroacetophenone. Cyclization then readily occurred in NaOMe/MeOH.22 Further reaction of **3** with methyl sulphate (Me_2SO_4) or benzylbromide (BnBr) in the presence of K_2CO_3 yielded compounds **6** and **7,** respectively.23 Compounds **4** and **5** were obtained from the corresponding diacetoxyacetophenones after bromination of the α-position of the ketone with triphenylmethylammonium tribromide, and then one-pot deprotection and cyclization in KOH/MeOH.24 The same approach was used for compound **9** , but with direct bromination of the 2-fluoro-6-hydroxyacetophenone with $CuBr_2$, then cyclization in a K_2CO_3 /DMF medium.25 Compound **8** was obtained from 3,5-difluorophenol, that was alkylated with chloroacetic acid. Subsequent chloration of the acid, followed by Friedel-Crafts cyclization, yielded the desired compound.26

Benzaldehyde derivatives were purchased from commercial sources, or synthesized using published approaches. 4-Cyclohexylbenzaldehyde was synthesized from cyclohexylbenzene in a formylation reaction, using hexamethylenetetramine in TFA.27 1-Butyl-1*H* -indole-3-carbaldehyde was obtained from indole-3-carbaldehyde and butyl bromide, using NaH in DMF.

Results and Discussion

An enzyme assay was used to assess the inhibition of the polymerase activity of a purified RdRp (non-structural 5B [NS5B] protein), deleted of its 21 C-terminal amino acids in order to ensure solubility (HCV-NS5BΔ21). This assay measures the amount of double-stranded RNA synthesized in the presence of HCV-NS5BΔ21, a homopolymeric RNA template and ATP, as previously described. 28 Initial screening was performed at an aurone concentration of 20 μM. The naturally occurring aureusidin (**1,** Figure 1) was found to be a potent inhibitor of HCV-NS5BΔ21 enzyme activity (85% inhibition at 20 μM, IC_{50} = 5.4±1.9 μM). This compound served as a “hit” for our subsequent investigations. As most naturally occurring aurones are hydroxylated at positions 4, 6, 2′, 3′ and 4′, we first investigated aurones bearing one, two, three or four hydroxyl groups at the above-mentioned positions (Table 1). The first structure-activity relationship study, undertaken to identify important substituents and their positions in the aurone core structure, showed that 4′ was the most important position for substitutions in the B-ring, whereas the most promising substituents in the A-ring were 4- and 6-hydroxyl groups (Table 1).

The next step was to identify the aurone binding site in HCV RdRp. Four sites, Thumb Pocket I and II and Palm Pockets I and II, have been identified as putative binding sites for non-nucleoside allosteric inhibitors of HCV RdRp (Figure 2). Amino acid substitutions at these sites have been shown to reduce susceptibility to the corresponding drugs. In order to determine if aurones target one of these sites, we examined whether these substitutions reduced HCV RdRp susceptibility to aurones in our cell-free enzyme assay. For this purpose we engineered HCV-NS5BΔ21 carrying the P495L, M423T, C316Y or H95Q substitutions, that are known to confer resistance to Thumb Pocket I, Thumb Pocket II, Palm Pocket I and Palm Pocket II inhibitors, respectively. The experiments were carried out with 4,4′ -dihydroxyaurone (aurone **11**). The IC_{50} s of aurone **11** for each HCV-NS5BΔ21 mutant were compared with the IC_{50} for wild-type HCV-NS5BΔ21, and the results were recorded as fold differences (Figure 3). A benzimidazole inhibitor of HCV RdRp, known to target

Thumb Pocket I, was used as a control. As expected, this latter compound was 10-fold less potent against HCV-NS5BΔ21-P495L than against HCV-NS5BΔ21-Wt, whereas its activity was not affected by the other amino acid substitutions. The inhibitory activity of aurone **11** was therefore only affected by the P495L substitution, indirectly suggesting that this compound binds to (or close to) Thumb Pocket I.

We used the findings reported in Table 1 and mutagenesis experiments to examine the structure-activity relationship of aurones. Various substituents were used to determine the influence of physico-chemical parameters, including hydrogen bond donor potential, electronegativity, sterical effects and lipophilicity. Molecular docking was used throughout the decision process to identify the relevant substituents and their positions. The results are shown in Table 2 . The presence of a hydroxyl group at position 4 appeared to be crucial. Replacement of the catechol moiety (B-ring) of **1** with a resorcinol in **34** did not affect inhibitory potency. Deletion of one of the B-ring hydroxyl groups of **34** yielded the less active derivative **12,** while suppression of both B-ring hydroxyl groups led to even less active aurones. Table 2 also indicates that the presence of two hydroxyl groups, at positions 4 and 6, instead of only one at position 4, slightly increased the activity.

Based on the reported X-ray crystallographic structure of genotype 1b HCV RdRp complexed with a tetracyclic indole-based inhibitor binding to Thumb Pocket I (PDB ID: 2dxs),29 docking calculations were used to rationalize the observed activities. The best poses generated with Autodock software for compound **1** showed that Arginine 503 anchored the molecule, forming two hydrogen bonds with the 4-hydroxyl group and the ketone of the aurone. Glycine 493 allowed the formation of a third hydrogen bond with the 6-hydroxy group. These findings potentially explained the slightly better activities of 4,6-dihydroxyaurones compared with 4-hydroxyaurones. At the B-ring, the 3′- and 4′-hydroxyl groups of **1** allowed the formation of two additional chelating hydrogen bonds with the carbonyl group of Leucine 392 inside the hydrophobic pocket, as shown in Figure 4A . These findings explain the strong impact of hydroxy groups at these positions.

Replacement of the 4′-hydroxyl group by its isosteric, fluorine atom led to the slightly less active derivative **42** , suggesting that hydrogen bonding plays a minor role in this region of the inhibitor. The introduction of methoxy groups instead of hydroxy groups at the B-ring led to compounds with similar (compounds **24** , **35**) or weaker (compounds **26** , **36**) activities, indicating that, in some cases, the insertion of more hydrophobic and bulky substituents is well tolerated. Conversely, methoxylation of the hydroxyl groups at the A-ring, or replacement of these groups by fluoro substituents, led to a marked fall in inhibitory activity (compounds **13** , **20** , **47** , **48**).

Given the strongly hydrophobic environment of Thumb Pocket I and the results of our initial docking studies, we tested the effect of adding hydrophobic substituents at the B-ring, especially at position 4′, on interactions with the pocket. The introduction of methyl and ethyl groups at the B-ring resulted in better inhibitory activity (compounds **23** and **37**). Thus, alkyl substituents were grafted at position 4′. The introduction of a *n* -butyl or a cyclohexyl group provided the best inhibitory activity (compounds **39** and **40**), indicating that both highly lipophilic and bulky substituents at the C-4′ position were associated with the strongest activities.

Overall, we identified the following pharmacophoric elements as crucial in the inhibitory activity of aurones on HCV RdRp: *a)* a hydroxyl group at position 4, or a dihydroxyl group at positions 4 and 6; and *b)* a hydrophobic and bulky substituent at position 4′ of the B-ring. We also found that replacement of the cyclohexyl by a 4-methylpiperazine in **31** was tolerated. Molecular docking of aurones **39** and **40** , based on the known crystallographic structure of genotype 1b HCV RdRp complexed with a tetracyclic indole-based inhibitor binding to Thumb Pocket I (PDB ID: 2dxs),29 showed strong fitting of the 4′-*n* -butyl and 4′-cyclohexyl moieties into the pocket. Further virtual docking screening suggested that aurone analogues in which the B-ring is replaced by a substituted indole moiety are potential ligands.

In models built with GOLD and Autodock software, the best poses were obtained for *N* -alkylindole analogues (*Z*)-2-(*N* -alkylindolyl)methylene)-4,6-dihydroxybenzofuran-3(2*H*)-one). Arginine 503 was found to anchor the molecule in a similar way to compound **1,** whereas hydrophobic residues (especially Leucine 492, Valine 37, Alanine 393, Leucine 392, Alanine 396, Alanine 395, Phenylalanine 429 and Isoleucine 424), forming two pockets, maintained both the indole part and the *N* -alkyl substituent of the ligand. This explains the positive effect of 4′-hydrophobic substituents. Compound **51** , which belongs to this group of indole derivatives, had a highly discriminant Goldscore of 52.4 (36.9 to 43.6 for compounds **23** , **24** , **27** , **28** , **29** , **39** and **40**). The fixation mode of compound **51** , which had the strongest inhibitory activity (IC_{50} = 2.2 μM), is shown in Figure 4B . These findings validated the docking results and confirmed the inhibitory potential of this group of indole derivatives.

Overall, the results obtained with weak inhibitors were, as expected, difficult to interpret, but the geometries of poses obtained with more efficient compounds were homogeneous and relevant. The strong activities of both 4′-hydroxyl and 4′-hydrophobic compounds could be explained by the good fit of these two subclasses of aurones in the pocket. Compounds **1** and **51** were representative of the hypothetic dual mode of aurone binding to Thumb Pocket I of HCV RdRp.

All the reported compounds were evaluated for cytotoxicity, based on the MTT assay with two human cell lines (HuH7 and HEK293) (data not shown). No cell death was observed at effective concentrations.

Conclusion

We investigated the capacity of aurones, non-toxic natural compounds, to inhibit HCV RdRp. Aureusidin potently inhibited the activity of this enzyme. By using classical medicinal chemistry, we identified positions and substituents that are crucial for the inhibitory activity of aurones on RdRp. A combination of site-directed mutagenesis and molecular modeling was successfully used to understand and optimize aurones' anti-HCV activity. Our findings constitute a milestone in the development of new non-nucleoside inhibitors of HCV RdRp. Studies are underway to synthesize more active compounds warranting preclinical evaluation.

Experimental section

Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a Brüker AC-400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts (δ) are reported in ppm relative to Me_4 Si (internal standard). Electrospray ionization ESI mass spectra were acquired by the Analytical Department of Grenoble University on an Esquire 300 Plus Bruker Daltonis instrument with a nanospray inlet. Elemental analyses were performed by the Analytical Department of Grenoble University. Thin-layer chromatography (TLC) used Merck silica gel F-254 plates (thickness 0.25 mm). Flash chromatography used Merck silica gel 60, 200–400 mesh. All solvents were distilled prior to use. Unless otherwise stated, reagents were obtained from commercial sources and were used without further purification. Compounds **2** to **9** were prepared with published procedures.22 –26

General procedure A for the preparation of (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivatives

To a solution of a benzofuran-3(2*H*)-one derivative in methanol (15 mL/mmol) were added an aqueous solution of potassium hydroxide (50%, 1.5 mL/mmol) and a benzaldehyde derivative (1.5 equivalents). The solution was then refluxed until TLC showed complete disappearance of the starting materials (1 to 18 hours). After cooling, the mixture was concentrated under reduced pressure, then the residue was diluted in water (50 mL/mmol), and an aqueous solution of hydrochloric acid (1N) was added to adjust the pH to 2–3. The mixture was extracted with ethyl acetate or dichloromethane and washed with water and brine. The combined organic layers were dried over magnesium sulfate and filtered, and the filtrate was concentrated under reduced pressure to give the crude compound.

General procedure B for the preparation of (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivatives

To a solution of a benzofuran-3(2*H*)-one derivative in ethanol (4 mL/mmol) were added an aqueous solution of potassium hydroxide (50%, 5 mL/mmol) and a benzaldehyde derivative (2 equivalents). The solution was then refluxed until TLC showed complete disappearance of the starting materials (2 to 5 hours). After cooling, the mixture was concentrated under reduced pressure, then the residue was diluted in water (50 mL/mmol), and an aqueous solution of hydrochloric acid (1N) was added to adjust the pH to 2–3. The mixture was extracted with ethyl acetate or dichloromethane and washed with water and brine. The combined organic layers were dried over magnesium sulfate and filtered, and the filtrate was concentrated under reduced pressure to give the crude compound.

General procedure C for the preparation of (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivatives

To a solution of a benzofuran-3(2*H*)-one derivative in anhydrous dichloromethane (20 mL/mmol) were added a benzaldehyde derivative (1.5 equivalents) and aluminium oxide (4000 mg/mmol). The suspension was stirred under argon overnight, and the solid was removed by filtration. The filtrate was distilled under reduced pressure to give the crude compound.

General procedure D for the preparation of (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivatives

To a solution of a (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivative in anhydrous dichloromethane (10 mL/mmol) was added pure boron tribromide (20 equivalents) at 0°C. The solution was then stirred at room temperature until TLC showed complete disappearance of the starting materials (24 to 72 hours). Cold water was then added and the suspension was extracted three times with ethyl acetate, and washed with water and brine. The combined organic layers were dried over magnesium sulfate and filtered, and the filtrate was concentrated under reduced pressure to give the crude compound.

(*Z*)-2-(3,4-dihydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (1)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(3,4-dimethoxy-benzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**52**), and was washed three times with distilled water to yield a yellow solid, which was analytically pure and used without further purification (61%). m.p. > 260°C (decomposition). ^{1}H NMR (400 MHz, MeOD) δ 7.47 (d, 1H, *J* = 1.4 Hz, $H_{2'}$), 7.18 (dd, 1H, *J* = 8.2 Hz, *J* = 1.4 Hz, $H_{6'}$), 6.82 (d, 1H, *J* = 8.2 Hz, $H_{5'}$), 6.56 (s, 1H, -CH=), 6.19 (s, 1H, H_7), 6.01 (s, 1H, H_5). ^{13}C NMR (100 MHz, MeOD) δ 178.9, 167.4, 166.9, 158.1, 147.4, 145.8, 145.4, 123.9, 123.6, 117.5, 115.9, 109.5, 102.8, 97.5, 90.2. MS (ESI) *m/z* 287 $(M+H)^+$, 309 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_6 \cdot 1.3H_2O$: C, 57.57, H, 4.05. Found: C, 57.12, H, 3.81.

(*Z*)-2-(4-hydroxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (10)

The crude product was prepared according to general procedure A starting from 6-hydroxybenzofuran-3(2*H*)-one (**2**) and 4-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure bright yellow crystals (77%). m.p. 294–296°C. ^{1}H NMR (400 MHz, d_6 -DMSO) δ 11.14 (br s, 1H, OH), 10.14 (br s, 1H, OH), 7,82 (d, 2H, *J* = 8.7 Hz, $H_{2',6'}$), 7.60 (d, 1H, *J* = 8.4 Hz, H_4), 6.88 (d, 2H, *J* = 8.7 Hz, $H_{3',5'}$), 6.78 (d, 1H, *J* = 1.9 Hz, H_7), 6.73 (s, 1H, -CH=), 6.70 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.9 Hz, H_5). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 181.2, 167.4, 166.0, 159.2, 145.6, 133.2, 125.6, 123.0, 116.0, 113.1, 112.8, 111.3, 98.4. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ½H_2O$: C, 68.44, H, 4.18. Found: C, 68.28, H, 4.11.

(*Z*)-2-(4-hydroxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (11)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure orange crystals (37%). m.p. 245–247°C. ^{1}H NMR (400 MHz, d_6 -DMSO) δ 11.05 (s, 1H, OH), 10.13 (s, 1H, OH), 7.81 (d, 2H, *J* = 8.4 Hz, $H_{2',6'}$), 7.51 (t, 1H, *J* = 8.1 Hz, H_6), 6.88 (d, 2H, *J* = 8.4 Hz, $H_{3',5'}$), 6.84 (d, 1H, *J* = 8.1 Hz, H_7), 6.70 (s, 1H, -CH=), 6.61 (d, 1H, *J* = 8.1 Hz, H_5). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 181.1, 165.7, 159.2, 156.9, 144.9, 138.2, 133.2, 123.1, 116.0, 110.9, 110.3, 109.4, 102.4. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot 0.1H_2O$: C, 70.37, H, 3.99. Found: C, 70.10, H, 3.96.

(*Z*)-2-(4-hydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (12)

To a solution of 4-hydroxybenzaldehyde (147 mg, 1.21 mmol) in methanol (10 mL) was added concentrated sulfuric acid (64.2 µL, 1.21 mmol), and the solution was stirred at room temperature for 30 minutes. 4,6-Dihydroxybenzofuran-3(2*H*)-one (**3** , 200 mg, 1.21 mmol) was then added, and the solution was refluxed for 4 hours. After cooling, the mixture was concentrated under reduced pressure, then the residue was diluted in water (100 mL), extracted with ethyl acetate and washed with water and brine. The combined organic layers were dried over magnesium sulfate and filtered, and the filtrate was concentrated under reduced pressure to give the crude compound, which was purified by column chromatography on silica gel (eluent: ethyl acetate 3/cyclohexane 2) to yield a pure yellow solid (17%). m.p. > 295°C (decomposition). ^{1}H NMR (400 MHz, d_6 -DMSO) δ 10.84 (br s, 1H, OH), 10.03 (br s, 1H, OH), 7.75 (d, 2H, *J* = 8.7 Hz, $H_{2',6'}$), 6.86 (d, 2H, *J* = 8.7 Hz, $H_{3',5'}$), 6.54 (s, 1H, -CH=), 6.20 (d, 1H, *J* = 1.6 Hz, H_7), 6.06 (d, 1H, *J* = 1.6 Hz, H_5). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 179.1, 167.6, 167.1, 158.8, 158.2, 146.0, 132.8, 123.4, 116.0, 109.1, 102.9, 97.7, 90.5. MS (ESI) *m/z* 271 $(M+H)^+$, 293 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_5$: C, 66.67, H, 3.73. Found: C, 66.43, H, 3.83.

(*Z*)-2-(4-hydroxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (13)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxy-benzofuran-3(2*H*)-one (**6**) and 4-hydroxybenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (63%). m.p. > 275°C (decomposition). ^{1}H NMR (400 MHz, d_6 -DMSO) δ 10.11 (s, 1H, OH), 7.79 (d, 2H, *J* = 8.6 Hz, $H_{2',6'}$), 6.87 (d, 2H, *J* = 8.6 Hz, $H_{3',5'}$), 6.68 (d, 1H, *J* = 1.1 Hz, H_7), 6.65 (s, 1H, -CH=), 6.33 (d, 1H, *J* = 1.1 Hz, H_5), 3.91 (s, 3H, OMe), 3.88 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 178.9, 168.6, 167.9, 159.1, 158.8, 145.6, 133.0, 123.1, 116.0, 110.4, 104.3, 94.3, 89.7, 56.4, 56.1. MS (ESI) *m/z* 299 $(M+H)^+$, 321 $(M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_5$: C, 68.46, H, 4.74. Found: C, 68.13, H, 4.78.

(*Z*)-2-(3-hydroxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (14)

The crude product was prepared according to general procedure A starting from 6-hydroxybenzofuran-3(2*H*)-one (**2**) and 3-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure yellow crystals (86%). m.p. 272–274°C. ^{1}H NMR (400 MHz, d_6 -DMSO) δ 11.27 (br s, 1H, OH), 9.69 (br s, 1H, OH), 7.63 (d, 1H, *J* = 8.4 Hz, H_4), 7.36 (m, 2H, $H_{2',6'}$), 7.28 (t, 1H, *J* = 7.6 Hz, $H_{5'}$), 6.84 (d, 1H, *J* = 7.6 Hz, $H_{4'}$), 6.78 (s, 1H, H_7), 6.72 (d, 1H, *J* = 8,4 Hz, H_5), 6.69 (s, 1H, -CH=). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 181.5, 167.9, 166.6, 157.6, 147.3, 133.1, 129.9, 126.1, 122.4, 117.3, 117.1, 113.1, 112.8, 110.7, 98.5. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ¼H_2O$: C, 69.63, H, 4.06. Found: C, 69.47, H, 3.94.

(*Z*)-2-(3-hydroxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (15)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 3-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure yellow crystals (55%). m.p. 207–209°C. 11.21 (br s, 1H, OH), 9.69 (br s, 1H, OH), 7.54 (t, 1H, *J* = 8.2 Hz, H_6), 7.36 (m, 2H, $H_{2',6'}$), 7.28 (t, 1H, *J* = 7.8 Hz, $H_{5'}$), 6.84 (m, 2H, $H_{4',7}$), 6.66 (s, 1H, -CH=), 6.64 (d, 1H, *J* = 8.2 Hz, H_5). ^{13}C NMR (100 MHz, d_6 -DMSO) δ 181.4, 166.0, 157.6, 157.2, 146.5, 138.7, 133.2, 129.9, 122.3, 117.2, 117.0, 110.7, 110.2, 109.0, 102.3. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4$: C, 70.87, H, 3.97. Found: C, 70.84, H, 4.74.

(*Z*)-2-(3-hydroxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (16)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxy-benzofuran-3(2*H*)-one (**6**) and 3-hydroxybenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (76%). m.p. > 250°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.65 (s, 1H, OH), 7.34 (m, 2H, $H_{2',6'}$), 7.27 (t, 1H, *J* = 8.0 Hz, $H_{5'}$), 6.83 (d, 1H, *J* = 8.0 Hz, $H_{4'}$), 6.67 (d, 1H, *J* = 1.2 Hz, H_7), 6.61 (s, 1H, -CH=), 6.35 (d, 1H, *J* = 1.2 Hz, H_5), 3.92 (s, 3H, OMe), 3.89 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.0, 169.0, 168.3, 159.0, 157.6, 147.2, 133.2, 129.9, 122.1, 117.3, 116.9, 109.8, 104.0, 94.4, 89.8, 56.5, 56.2. MS (ESI) *m/z* 299 $(M+H)^+$, 321 $(M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_5 \cdot H_2O$: C, 64.56, H, 5.06. Found: C, 64.31, H, 5.06.

(*Z*)-2-(2-hydroxybenzylidene)-6-hydroxybenzofuran-3(2*H*)-one (17)

The crude product was prepared according to general procedure A starting from 6-hydroxybenzofuran-3(2*H*)-one (**2**) and 2-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure yellow crystals (25%). m.p. > 250°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.21 (br s, 1H, OH), 10.35 (br s, 1H, OH), 8.09 (d, 1H, *J* = 7.0 Hz, $H_{6'}$), 7.62 (d, 1H, *J* = 8.4 Hz, H_4), 7.26 (t, 1H, *J* = 7.0 Hz, $H_{4'}$), 7.09 (s, 1H, -CH=), 6.94 (m, 2H, $H_{3',5'}$), 6.79 (d, 1H, *J* = 1.5 Hz, H_7), 6.72 (dd, 1H, *J* = 8.4 Hz, *J* = 1.5 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.4, 167.7, 166.3, 157.0, 146.8, 131.3, 130.9, 125.8, 119.6, 118.8, 115.6, 112.9, 104.6, 98.5. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ¼ H_2O$: C, 69.63, H, 4.06. Found: C, 69.81, H, 4.05.

(*Z*)-2-(2-hydroxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (18)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure yellow crystals (28%). m.p. > 250°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.10 (br s, 1H, OH), 10.35 (s, 1H, OH), 8.08 (d, 1H, *J* = 7.2 Hz, $H_{6'}$), 7.52 (t, 1H, *J* = 7.6 Hz, H_6), 7.24 (t, 1H, *J* = 7.2 Hz, $H_{4'}$), 7.06 (s, 1H, -CH=), 6.94 (m, 2H, $H_{3',5'}$), 6.86 (d, 1H, *J* = 7.6 Hz, H_7), 6.62 (d, 1H, *J* = 7.6 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.2, 165.8, 157.0, 157.0, 146.0, 138.4, 131.2, 130.8, 119.6, 118.9, 115.6, 110.4, 109.1, 104.1, 102.4. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ¼H_2O$: C, 69.63, H, 4.06. Found: C, 69.19, H, 3.89.

(*Z*)-2-(2-hydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (19)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(2-hydroxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**16**), and was purified by column chromatography on silica gel (eluent: ethyl acetate 1/cyclohexane 1) to yield a pure yellow solid (66%). m.p. 262–264°C. ^{1}H NMR (400 MHz, d_6-acetone) δ 8.12 (d, 1H, *J* = 7.2 Hz, $H_{6'}$), 7.17 (m, 1H, $H_{4'}$), 7.12 (s, 1H, -CH=), 6.90 (m, 2H, $H_{3'}$, $H_{5'}$), 6.29 (d, 1H, *J* = 2.0 Hz, H_7), 6.08 (d, 1H, *J* = 2.0 Hz, H_5). ^{13}C NMR (100 MHz, d_6-acetone) δ 180.9, 167.6, 167.5, 157.9, 156.7, 147.5, 131.2, 130.9, 120.0, 119.6, 115.5, 103.6, 103.4, 97.7, 91.2. MS (ESI) *m/z* 271 $(M+H)^+$. Anal. Calcd for $C_{15}H_{10}O_5 \cdot ⅔H_2O$: C, 65.22, H, 3.86. Found: C, 65.37, H, 4.61.

(*Z*)-2-(2-hydroxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (20)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxy-benzofuran-3(2*H*)-one (**6**) and 2-hydroxybenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (51%). m.p. > 250°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.31 (s, 1H, OH), 8.06 (d, 1H, *J* = 7.8 Hz, $H_{6'}$), 7.24 (t, 1H, *J* = 7.8 Hz, $H_{4'}$), 7.01 (s, 1H, -CH=), 6.94 (d, 1H, *J* = 7.8 Hz, $H_{3'}$), 6.92 (t, 1H, *J* = 7.8 Hz, $H_{5'}$), 6.71 (d, 1H, *J* = 1.4 Hz, H_7), 6.34 (d, 1H, *J* = 1.4 Hz, H_5), 3.91 (s, 3H, OMe), 3.89 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.0, 168.7, 168.0, 158.8, 156.9, 146.7, 131.1, 130.6, 119.4, 118.8, 115.6, 104.0, 103.6, 94.3, 89.8, 56.4, 56.0. MS (ESI) *m/z* 299 $(M+H)^+$, 321 $(M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_4$: C, 68.46, H, 4.74. Found: C, 68.39, H, 4.77.

(*Z*)-2-benzylidene-4-hydroxybenzofuran-3(2*H*)-one (21)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and benzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane) to yield a pure yellow solid (72%). m.p. 148°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.19 (s, 1H, OH), 7.95 (d, 2H, *J* = 7.2 Hz, $H_{2',6'}$), 7.54 (t, 1H, *J* = 8.1 Hz, H_6), 7.46 (m, 3H, $H_{3',4',5'}$), 6.87 (d, 1H, *J* = 8.1 Hz, H_7), 6.77 (s, 1H, -CH=), 6.64 (d, 1H, *J* = 8.1 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.4, 166.1, 157.2, 146.6, 138.8, 132.2, 131.0, 129.6, 129.0, 110.7, 109.9, 109.0, 102.5. MS (ESI) *m/z* 239 $(M+H)^+$, 261 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_3 \cdot ⅔H_2O$: C, 72.00, H, 4.53. Found: C, 71.99, H, 4.15.

(*Z*)-2-(2,4-dihydroxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (22)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(2,4-dimethoxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (**24**), and was washed three times with distilled water to yield a yellow solid, which was analytically pure and used without further purification (61%). m.p. > 230°C (decomposition). ^{1}H NMR (400 MHz, d_6

-DMSO) δ 10.94 (s, 1H, OH), 10.29 (s, 1H, OH), 10.02 (s, 1H, OH), 7.96 (d, 1H, *J* = 8.2 Hz, $H_{6'}$), 7.48 (t, 1H, *J* = 8.0 Hz, H_6), 7.03 (s, 1H, -CH=), 6.83 (d, 1H, *J* = 8.0 Hz, H_7), 6.59 (d, 1H, *J* = 8.0 Hz, H_5), 6.40 (m, 2H, $H_{3',5'}$). ^{13}C NMR (400 MHz, d_6-DMSO) δ 181.0, 165.6, 160.9, 159.1, 156.8, 144.3, 137.8, 132.4, 110.7, 110.1, 109.7, 108.3, 105.6, 102.5, 102.3. MS (ESI) *m/z* 271 $(M+H)^+$, 293 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_5 \cdot H_2O$: C, 62.50, H, 4.17. Found: C, 62.64, H, 3.70.

(*Z*)-2-(2,4-dimethylbenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (23)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2,4-dimethylbenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (76%). m.p. 193–194°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.14 (s, 1H, OH), 8.01 (d, 1H, *J* = 7.9 Hz, $H_{6'}$), 7.52 (t, 1H, *J* = 8.1 Hz, H_6), 7.12 (m, 2H, $H_{3',5'}$), 6.84 (d, 1H, *J* = 8.1 Hz, H_7), 6.78 (s, 1H, -CH=), 6.63 (d, 1H, *J* = 8.1 Hz, H_5), 2.40 (s, 3H, Me), 2.29 (s, 3H, Me). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.2, 166.0, 157.1, 146.2, 139.4, 138.5, 138.4, 131.3, 130.3, 127.6, 127.1, 110.6, 109.1, 106.5, 102.4, 21.0, 19.6. MS (ESI) *m/z* 267 $(M+H)^+$, 289 $(M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_3 \cdot \frac{1}{3}H_2O$: C, 75.00, H, 5.39. Found: C, 74.96, H, 5.35.

(*Z*)-2-(2,4-dimethoxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (24)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2,4-dimethoxybenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (76%). m.p. 234–235°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.05 (s, 1H, OH), 8.11 (d, 1H, *J* = 8.6 Hz, $H_{6'}$), 7.51 (t, 1H, *J* = 8.0 Hz, H_6), 6.99 (s, 1H, -CH=), 6.84 (d, 1H, *J* = 8.0 Hz, H_7), 6.70 (d, 1H, *J* = 8.6 Hz, $H_{5'}$), 6.66 (s, 1H, $H_{3'}$), 6.62 (d, 1H, *J* = 8.0 Hz, H_5), 3.90 (s, 3H, OMe), 3.84 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.2, 165.8, 162.4, 159.8, 157.0, 145.3, 138.3, 132.2, 113.2, 110.5, 109.4, 106.7, 103.9, 102.6, 98.3, 56.0, 55.6. MS (ESI) *m/z* 299 $(M+H)^+$, 321 $(M+Na)^+$, 619 $(2M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_5 \cdot \frac{1}{2}H_2O$: C, 66.44, H, 4.92. Found: C, 66.60, H, 4.88.

(*Z*)-2-(2,6-dimethoxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (25)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2,6-dimethoxybenzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane) to yield pure yellow solid (34%). m.p. 152–153°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br s, 1H, OH), 7.45 (t, 1H, *J* = 8.1 Hz, H_6), 7.34 (t, 1H, *J* = 8.4 Hz, $H_{4'}$), 7.09 (s, 1H, -CH=), 6.66 (d, 1H, *J* = 8.1 Hz, H_7), 6.61 (d, 2H, *J* = 8.4 Hz, $H_{3',5'}$), 6.58 (d, 1H, *J* = 8.1 Hz, H_5), 3.88 (s, 6H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.5, 165.1, 159.1, 156.7, 147.5, 139.2, 131.5, 110.1, 109.9, 109.3, 106.5, 104.0, 103.5, 56.0. MS (ESI) *m/z* 299 $(M+H)^+$, 321 $(M+Na)^+$, 619 $(2M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_5 \cdot \frac{1}{2}H_2O$: C, 66.45, H, 4.88. Found: C, 66.45, H, 4.78.

(*Z*)-2-(2,4,6-trimethoxybenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (26)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2,4,6-trimethoxybenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (71%). m.p. 139–140°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.00 (s, 1H, OH), 7.46 (t, 1H, *J* = 8.1 Hz, H_6), 6.68 (d, 1H, *J* = 8.1 Hz, H_7), 6.66 (s, 1H, -CH=), 6.57 (d, 1H, *J* = 8.1 Hz, H_5), 6.31 (s, 2H, $H_{3,5}$), 3.84 (s, 3H, OMe), 3.83 (s, 6H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.1, 165.9, 162.8, 159.5, 156.9, 146.1, 138.4, 109.9, 109.5, 103.4, 102.2, 91.1, 55.9, 55.5. MS (ESI) *m/z* 329 $(M+H)^+$, 351 $(M+Na)^+$, 679 $(2M+Na)^+$. Anal Calcd for $C_{18}H_{16}O_6 \cdot \frac{1}{3}H_2O$: C, 64.67, H, 4.99. Found: C, 64.58, H, 5.03.

(*Z*)-2-(4-ethylbenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (27)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-ethylbenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (50%). m.p. 141–142°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.16 (s, 1H, OH), 7.86 (d, 2H, *J* = 8.0 Hz, $H_{2',6'}$), 7.53 (t, 1H, *J* = 8.1 Hz, H_6), 7.32 (d, 2H, *J* = 8.0 Hz, $H_{3',5'}$), 6.86 (d, 1H, *J* = 8.1 Hz, H_7), 6.74 (s, 1H, -CH=), 6.64 (d, 1H, *J* = 8.1 Hz, H_5), 2.64 (q, 2H, *J* = 7.5 Hz, PhC*H*$_2$CH$_3$), 1.19 (t, 3H, *J* = 7.5 Hz, PhCH$_2$C*H*$_3$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.3, 166.0, 157.1, 146.2, 145.8, 138.6, 131.1, 129.6, 128.4, 110.6, 110.1, 109.1, 102.4, 28.2, 15.3. MS (ESI) *m/z* 267 $(M+H)^+$, 289 $(M+Na)^+$. Anal. Calcd for $C_{17}H_{14}O_3 \cdot \frac{1}{8}H_2O$: C, 76.04, H, 5.31. Found: C, 75.93, H, 5.26.

(*Z*)-2-(4-isopropylbenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (28)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-isopropylbenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (44%). m.p. 126–127°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.16 (s, 1H, OH), 7.86 (d, 2H, *J* = 7.9 Hz, $H_{2',6'}$), 7.54 (t, 1H, *J* = 8.1 Hz, H_6), 7.36 (d, 2H, *J* = 7.9 Hz, $H_{3',5'}$), 6.86 (d, 1H, *J* = 8.1 Hz, H_7), 6.74 (s, 1H, -CH=), 6.64 (d, 1H, *J* = 8.1 Hz, H_5), 2.92 (sept., 1H, *J* = 6.8 Hz, PhC*H*$(CH_3)_2$), 1.21 (d, 6H, *J* = 6.8 Hz, PhCH(C*H*$_3$)$_2$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.3, 166.0, 157.1, 150.4, 146.2, 138.6, 131.2, 129.8, 127.0, 110.6, 110.1, 109.1,

102.4, 33.5, 23.6. MS (ESI) *m/z* 281 $(M+H)^+$, 303 $(M+Na)^+$. Anal. Calcd for $C_{18}H_{16}O_3 \cdot ¼H_2O$: C, 75.92, H, 5.79. Found: C, 75.55, H, 5.62.

(*Z*)-2-(4-butylbenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (29)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-butylbenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (56%). m.p. 121–122°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.15 (s, 1H, OH), 7.85 (d, 2H, *J* = 7.9 Hz, $H_{2',6'}$), 7.53 (t, 1H, *J* = 8.1 Hz, H_6), 7.30 (d, 2H, *J* = 7.9 Hz, $H_{3',5'}$), 6.86 (d, 1H, *J* = 8.1 Hz, H_7), 6.74 (s, 1H, -CH=), 6.63 (d, 1H, *J* = 8.0 Hz, H_5), 2.61 (t, 2H, *J* = 7.4 Hz, $PhCH_2CH_2CH_2CH_3$), 1.56 (quint., 2H, *J* = 7.4 Hz, $PhCH_2CH_2CH_2CH_3$), 1.30 (sext., 2H, *J* = 7.4 Hz, $PhCH_2CH_2CH_2CH_3$), 0.89 (t, 3H, *J* = 7.4 Hz, $PhCH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.3, 166.0, 157.1, 146.2, 144.5, 138.6, 131.1, 129.6, 129.0, 110.6, 110.2, 109.1, 102.4, 34.8, 32.9, 21.8, 13.8. MS (ESI) *m/z* 295 $(M+H)^+$, 317 $(M+Na)^+$. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53, H, 6.17. Found: C, 77.32, H, 6.33.

(*Z*)-2-(4-tertiobutylbenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (30)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-tertiobutylbenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (61%). m.p. 167–168°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.16 (s, 1H, OH), 7.86 (d, 2H, *J* = 8.0 Hz, $H_{2',6'}$), 7.54 (t, 1H, *J* = 8.1 Hz, H_6), 7.49 (d, 2H, *J* = 8.0 Hz, $H_{3',5'}$), 6.85 (d, 1H, *J* = 8.1 Hz, H_7), 6.74 (s, 1H, -CH=), 6.64 (d, 1H, *J* = 8.1 Hz, H_5), 1.28 (s, 9H, $Ph(CH_3)_3$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.3, 166.0, 157.2, 152.6, 146.3, 138.6, 130.9, 129.4, 125.8, 110.6, 110.0, 109.2, 102.4, 34.6, 30.9. MS (ESI) *m/z* 295 $(M+H)^+$, 317 $(M+Na)^+$. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53, H, 6.17. Found: C, 77.22, H, 6.24.

(*Z*)-2-(4-(4-methylpiperazin-1-yl)benzylidene)-4-hydroxybenzofuran-3(2*H*)-one (31)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 4-(4-methylpiperazin-1-yl)benzaldehyde, and was washed three times with distilled water to yield an orange solid, which was the analytically pure hydrochloride salt, used without further purification (71%). m.p. > 260°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.14 (s, 1H, OH), 11.14 (br s, 1H, NH^+) 7.85 (d, 2H, *J* = 8.4 Hz, $H_{2',6'}$), 7.51 (t, 1H, *J* = 8.1 Hz, H_6), 7.10 (d, 2H, *J* = 8.4 Hz, $H_{3',5'}$), 6.84 (d, 1H, *J* = 8.1 Hz, H_7), 6.72 (s, 1H, -CH=), 6.66 (d, 1H, *J* = 8.1 Hz, H_5), 4.02 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.24 (m, 2H, CH_2), 3.13 (m, 2H, CH_2), 2.80 (s, 3H, NMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 180.8, 165.5, 156.9, 149.9, 145.0, 137.9, 132.4, 122.8, 115.1, 110.6, 110.3, 109.3, 102.2, 51.7, 44.2, 41.8. MS (ESI) *m/z* 337 $(M+H)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot HCl \cdot 1.75H_2O$: C, 57.14, H, 5.83, N, 6.67. Found: C, 57.22, H, 5.95, N, 6.76.

(*Z*)-2-(2-fluorobenzylidene)-4-hydroxybenzofuran-3(2*H*)-one (32)

The crude product was prepared according to general procedure A starting from 4-hydroxybenzofuran-3(2*H*)-one (**5**) and 2-fluorobenzaldehyde, and was recrystallized from methanol to yield pure yellow crystals (30%). m.p. 162–163°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.29 (s, 1H, OH), 8.21 (m, 1H, $H_{6'}$), 7.56 (t, 1H, *J* = 8.1 Hz, H_6), 7.50 (m, 1H, $H_{3'}$), 7.35 (m, 2H, $H_{4',5'}$), 6.87 (d, 1H, *J* = 8.1 Hz, H_7), 6.75 (s, 1H, -CH=), 6.66 (d, 1H, *J* = 8.1 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.1, 166.1, 160.6 (d, *J* = 251.6 Hz), 157.4, 147.6, 139.1, 131.7 (d, *J* = 8.0 Hz), 131.1, 125.2, 119.9 (d, *J* = 11.5 Hz), 115.8 (d, *J* = 21.5 Hz), 111.0, 108.8, 102.5, 99.8 (d, *J* = 7.3 Hz). MS (ESI) *m/z* 257 $(M+H)^+$, 279 $(M+Na)^+$. Anal. Calcd for $C_{15}H_9FO_3 \cdot ⅔H_2O$: C, 67.16, H, 3.85. Found: C, 66.80, H, 3.58.

(*Z*)-2-benzylidene-4,6-dihydroxybenzofuran-3(2*H*)-one (33)

The crude product was prepared according to general procedure D starting from (*Z*)-2-benzylidene-4,6-dimethoxy-benzofuran-3(2*H*)-one (**54**), and was purified by column chromatography on silica gel (eluent: toluene 9/methanol 1) to yield a pure yellow solid (33%). m.p. > 225°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.99 (br s, 2H, OH), 7.89 (d, 2H, *J* = 7.4 Hz, $H_{3',5'}$), 7.47 (m, 2H, $H_{2',6'}$), 7.39 (m, 1H, $H_{4'}$), 6.61 (s, 1H, -CH=), 6.23 (s, 1H, H_7), 6.08 (s, 1H, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 180.0, 167.8, 158.4, 147.7, 132.3, 130.6, 129.1, 128.9, 108.0, 102.4, 97.7, 90.6. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ⅔H_2O$: C, 67.67, H, 4.26. Found: C, 67.67, H, 4.15.

(*Z*)-2-(2,4-dihydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (34)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(2,4-dimethoxy-benzylidene)-4,6-dimethoxybenzo-furan-3(2*H*)-one (**53**), and was washed three times with distilled water to yield an orange solid, which was analytically pure and used without further purification (58%). m.p. > 270°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.77 (s, 1H, OH), 10.74 (s, 1H, OH), 10.16 (s, 1H, OH), 9.90 (s, 1H, OH), 7.89 (d, 1H, *J* = 8.4 Hz, $H_{6'}$), 6.88 (s, 1H, -CH=), 6.37 (m, 2H, $H_{3',5'}$), 6.18 (d, 1H, *J* = 1.6 Hz, H_7), 6.05 (d, 1H, *J* = 1.6 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.1, 167.4,

166.7, 160.3, 158.6, 158.0, 145.4, 132.0, 110.8, 108.1, 103.6, 103.1, 102.3, 97.5, 90.4. MS (ESI) *m/z* 287 $(M+H)^+$, 309 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_6 \cdot 1.1H_2O$: C, 58.86, H, 3.99. Found: C, 58.78, H, 3.98.

(*Z*)-2-(2,3-dimethoxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (35)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 2,3-dimethoxybenzaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure yellow solid (72%). m.p. 272–273°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.00 (s, 1H, OH), 10.94 (s, 1H, OH), 7.73 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz, $H_{6'}$), 7.19 (t, 1H, J = 8.0 Hz, $H_{5'}$), 7.12 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz, $H_{4'}$), 6.80 (s, 1H, -CH=), 6.21 (d, J = 1.6 Hz, 1H, H_7), 6.08 (d, J = 1.6 Hz, 1H, H_5), 3.84 (s, 3H, OMe), 3.80 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 178.9, 167.7, 167.4, 158.5, 152.5, 148.3, 147.8, 125.9, 124.4, 122.0, 114.0, 102.4, 101.4, 97.8, 90.6, 60.9, 55.8.

(*Z*)-2-(2,3,4-trimethoxybenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (36)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 2,3,4-trimethoxybenzaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure yellow solid (80%). m.p. 245–246°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.13 (s, 2H, OH), 7.87 (d, 1H, J = 7.6 Hz, $H_{6'}$), 6.97 (d, 1H, J = 7.6 Hz, $H_{5'}$), 6.72 (s, 1H, -CH=), 6.27 (s, 1H, H_7), 6.23 (s, 1H, H_5), 3.86 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.76 (s, 3H, OMe). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.0, 169.5, 169.5, 160.4, 156.6, 154.6, 149.3, 143.7, 127.9, 120.6, 110.6, 104.5, 103.7, 99.9, 92.5, 63.6, 62.5, 58.0.

(*Z*)-2-(4-ethylbenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (37)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 4-ethylbenzaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure yellow solid (83%). m.p. 237–238°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.94 (s, 2H, OH), 7.81 (d, 2H, J = 8.0 Hz, $H_{2',6'}$), 7.31 (d, 2H, J = 8.0 Hz, $H_{3',5'}$), 6.58 (s, 1H, -CH=), 6.22 (s, 1H, H_7), 6.08 (s, 1H, H_5), 2.64 (q, 2H, J = 7.6 Hz, CH_2CH_3), 1.18 (t, 3H, J = 7.6 Hz, CH_2CH_3). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.1, 169.8, 169.4, 160.4, 149.3, 147.3, 132.8, 131.8, 130.4, 110.3, 104.6, 99.7, 92.6, 30.1, 17.4. Anal. Calcd for $C_{17}H_{14}O_4 \cdot ¼H_2O$: C, 71.20, H, 5.06. Found: C, 71.47, H, 5.19.

(*Z*)-2-(4-isopropylbenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (38)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 4-isopropylbenzaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure yellow solid (88%). m.p. 229–230°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.94 (s, 2H, OH), 7.80 (d, 2H, J = 8.0 Hz, $H_{2',6'}$), 7.32 (d, 2H, J = 8.0 Hz, $H_{3',5'}$), 6.58 (s, 1H, -CH=), 6.23 (d, 1H, J = 2.0 Hz, H_7), 6.09 (d, 1H, J = 2.0 Hz, H_5), 2.89 (sept, 1H, J = 6.8 Hz, $CH(CH_3)_2$), 1.19 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 181.1, 169.8, 169.4, 160.4, 151.8, 149.4, 132.8, 132.0, 128.9, 110.3, 104.6, 99.7, 92.6, 35.4, 25.6. Anal. Calcd for $C_{18}H_{16}O_4 \cdot ¼H_2O$: C, 71.88, H, 5.49. Found: C, 71.99, H, 5.52.

(*Z*)-2-(4-butylbenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (39)

The crude product was prepared according general procedure D starting from (*Z*)-2-(4-butylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**44**), and was purified by column chromatography on silica gel (eluent: ethyl acetate 1/cyclohexane 1) to yield a pure yellow solid (52%). m.p. 221–222°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.95 (br s, 2H, OH), 7.79 (d, 2H, J = 8.1 Hz, $H_{2',6'}$), 7.29 (d, 2H, J = 8.1 Hz, $H_{3',5'}$), 6.57 (s, 1H, -CH=), 6.21 (s, 1H, H_7), 6.07 (s, 1H, H_5), 2.61 (t, 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 1.56 (quint., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 1.31 (sext., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 0.89 (t, 3H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.0, 167.8, 167.6, 158.5, 147.4, 143.9, 130.7, 129.9, 128.9, 108.3, 102.6, 97.8, 90.5, 34.8, 32.9, 21.8, 13.8. MS (ESI) *m/z* 311 $(M+H)^+$, 333 $(M+Na)^+$, 643 $(2M+Na)^+$. Anal. Calcd for $C_{19}H_{18}O_4 \cdot ⅓H_2O$: C, 72.15, H, 5.91. Found: C, 72.00, H, 5.83.

(*Z*)-2-(4-cyclohexylbenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (40)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(4-cyclohexylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**45**), and was purified by column chromatography on silica gel (eluent: ethyl acetate 1/cyclohexane 1) to yield a pure yellow solid (64%). m.p. > 215°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.98 (br s, 2H, OH), 7.79 (d, 2H, J = 8.3 Hz, $H_{2',6'}$), 7.31 (d, 2H, J = 8.3 Hz, $H_{3',5'}$), 6.56 (s, 1H, -CH=), 6.20 (d, 1H, J = 1.5 Hz, H_7), 6.07 (d, 1H, J = 1.5 Hz, H_5), 2.54 (m, 1H, *c*-Hex), 1.79 (m, 4H, *c*-Hex), 1.70 (m, 1H, *c*-Hex), 1.39 (m, 4H, *c*-Hex), 1.31 (m, 1H, *c*

-Hex). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.0, 167.8, 167.5, 158.5, 149.0, 147.4, 130.7, 130.0, 127.3, 108.2, 102.5, 97.7, 90.5, 43.7, 33.7, 26.3, 25.5. MS (ESI) *m/z* 337 $(M+H)^+$, 359 $(M+Na)^+$, 695 $(2M+Na)^+$. Anal. Calcd for $C_{21}H_{20}O_4 \cdot ½H_2O$: C, 73.03, H, 6.13. Found: C, 72.95, H, 6.01.

(*Z*)-2-(4-morpholinobenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (41)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 4-morpholinobenzaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure red solid (32%). m.p. 273–273°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.83 (s, 1H, OH), 10.79 (s, 1H, OH), 7.77 (d, 2H, *J* = 8.6 Hz, $H_{2',6'}$), 7.02 (d, 2H, *J* = 8.6 Hz, $H_{3',5'}$), 6.55 (s, 1H, -CH=), 6.20 (d, 1H, *J* = 0.8 Hz, H_7), 6.06 (d, 1H, *J* = 0.8 Hz, H_5), 3.74 (t, 4H, *J* = 4.8 Hz, CH_2O), 3.23 (t, 4H, *J* = 4.8 Hz, CH_2N). ^{13}C NMR (100 MHz, d_6-DMSO) δ 180.9, 169.4, 168.9, 160.1, 153.3, 147.9, 134.2, 124.4, 116.4, 111.3, 104.9, 99.6, 92.4, 67.9, 49.2. Anal. Calcd for $C_{19}H_{17}NO_5 \cdot ⅓H_2O$: C, 66.08, H, 5.12, N, 4.05. Found: C, 66.44, H, 5.54, N, 3.37.

(*Z*)-2-(4-fluorobenzylidene)-4,6-dihydroxybenzofuran-3(2*H*)-one (42)

The crude product was prepared according to general procedure D starting from (*Z*)-2-(4-fluorobenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**55**), and was purified by column chromatography on silica gel (eluent: dichloromethane 9/methanol 1) to yield a pure yellow solid (50%). m.p. > 270°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.00 (br s, 1H, OH), 10.97 (br s, 1H, OH), 7.96 (m, 2H, $H_{2',6'}$), 7.31 (m, 2H, $H_{3',5'}$), 6.64 (s, 1H, -CH=), 6.22 (d, 1H, *J* = 1.6 Hz, H_7), 6.08 (d, 1H, *J* = 1.6 Hz, H_5). ^{13}C NMR (100 MHz, d_6-DMSO) δ 179.0, 167.9, 167.5, 162.3 (d, *J* = 248.4 Hz), 158.5, 147.5, 132.9 (d, *J* = 8.2 Hz), 129.1, 116.0 (d, *J* = 21.7 Hz), 107.1, 102.5, 97.8, 90.7. MS (ESI) *m/z* 273 $(M+H)^+$, 295 $(M+Na)^+$. Anal. Calcd for $C_{15}H_9FO_4 \cdot ⅓H_2O$: C, 64.74, H, 3.48. Found: C, 64.58, H, 3.67.

(*Z*)-2-(2,4-dimethylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (43)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxy-benzofuran-3(2*H*)-one (**6**) and 2,4-dimethoxybenzaldehyde, and was recrystallized from methanol to yield pure pale yellow crystals (86%). m.p. 216°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, 1H, *J* = 8.0 Hz, $H_{6'}$), 7.10 (d, 1H, *J* = 8.0 Hz, $H_{5'}$), 7.06 (s, 1H, $H_{3'}$), 6.97 (s, 1H, -CH=), 6.37 (d, 1H, *J* = 1.7 Hz, H_7), 6.12 (d, 1H, *J* = 1.7 Hz, H_5), 3.95 (s, 3H, OMe), 3.90 (s, 3H, OMe), 2.46 (s, 3H, Me), 2.35 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 169.2, 169.0, 159.6, 147.8, 139.8, 139.0, 131.6, 131.0, 128.4, 127.2, 108.1, 105.6, 94.2, 89.4, 56.4, 56.3, 21.6, 20.4. MS (ESI) *m/z* 311 $(M+H)^+$, 333 $(M+Na)^+$, 643 $(2M+Na)^+$. Anal. Calcd for $C_{19}H_{18}O_4$: C, 73.54, H, 5.85. Found: C, 73.04, H, 5.92.

(*Z*)-2-(4-butylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (44)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and 4-butylbenzaldehyde, and was recrystallized from methanol to yield pure white crystals (75%). m.p. 153°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, *J* = 8.1 Hz, $H_{2',6'}$), 7.24 (d, 2H, *J* = 8.1 Hz, $H_{3',5'}$), 6.77 (s, 1H, -CH=), 6.38 (d, 1H, *J* = 1.7 Hz, H_7), 6.12 (d, 1H, *J* = 1.7 Hz, H_5), 3.95 (s, 3H, OMe), 3.91 (s, 3H, OMe), 2.65 (t, 2H, *J* = 7.6 Hz, PhCH_2 CH_2 CH_2 CH_3), 1.61 (quint., 2H, *J* = 7.6 Hz, $PhCH_2CH_2CH_2CH_3$), 1.37 (sext., 2H, *J* = 7.6 Hz, $PhCH_2CH_2CH_2CH_3$), 0.96 (t, 3H, *J* = 7.6 Hz, $PhCH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.0, 169.2, 169.1, 159.6, 147.7, 145.0, 131.3, 130.2, 129.1, 111.3, 105.5, 94.2, 89.4, 56.4, 56.3, 35.8, 33.6, 22.5, 14.1. MS (ESI) *m/z* 339 $(M+H)^+$, 361 $(M+Na)^+$, 699 $(2M+Na)^+$. Anal. Calcd for $C_{21}H_{22}O_4$: C, 74.54, H, 6.56. Found: C, 74.46, H, 6.33.

(*Z*)-2-(4-cyclohexylbenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (45)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and 4-cyclohexylbenzaldehyde, and was recrystallized from methanol to yield pure white crystals (78%). m.p. 174°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, *J* = 8.2 Hz, $H_{2',6'}$), 7.27 (d, 2H, *J* = 8.2 Hz, $H_{3',5'}$), 6.76 (s, 1H, -CH=), 6.37 (d, 1H, *J* = 1.6 Hz, H_7), 6.11 (d, 1H, *J* = 1.6 Hz, H_5), 3.94 (s, 3H, OMe), 3.90 (s, 3H, OMe), 2.53 (m, 1H, *c*-Hex), 1.86 (m, 4H, *c*-Hex), 1.76 (m, 1H, *c*-Hex), 1.42 (m, 4H, *c*-Hex), 1.35 (m, 1H, *c*-Hex). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 169.1, 169.0, 159.5, 150.0, 147.7, 131.4, 130.3, 127.5, 111.2, 105.5, 94.1, 89.4, 56.4, 56.3, 44.7, 34.4, 27.0, 26.2. MS (ESI) *m/z* 365 $(M+H)^+$, 751 $(2M+Na)^+$. Anal. Calcd for $C_{23}H_{24}O_4$: C, 75.81, H, 6.64. Found: C, 75.40, H, 6.78.

(*Z*)-2-(4-butylbenzylidene)-benzofuran-3(2*H*)-one (46)

The crude product was prepared according to general procedure C starting from benzofuran-3(2*H*)-one and 4-butylbenzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane 1/cyclohexane 1) to yield a pure pale yellow solid (44%). m.p. 77–78°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, 2H, *J* = 8.3 Hz, $H_{2',6'}$), 7.83 (d, 1H, *J* = 8.3 Hz, H_4), 7.67 (t, 1H, *J* = 8.3 Hz, H_6), 7.35 (d, 1H, *J* = 8.3 Hz, H_7), 7.30 (d, 2H, *J* = 8.3 Hz, $H_{3',5'}$), 7.24 (t, 1H, *J* = 8.3 Hz, H_5), 6.92 (s, 1H, -CH=), 2.68 (t, 2H, *J* = 7.5 Hz, PhC

$H_2CH_2CH_2CH_3$), 1.65 (quint., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 1.40 (sext., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 0.96 (t, 3H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.0, 166.2, 146.7, 145.7, 136.9, 131.8, 129.9, 129.3, 124.8, 123.6, 122.0, 113.7, 113.1, 35.9, 33.6, 22.6, 14.2. MS (ESI) *m/z* 279 $(M+H)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ¼H_2O$: C, 80.71, H, 6.55. Found: C, 80.96, H, 6.77.

(*Z*)-2-(4-butylbenzylidene)-4,6-difluorobenzofuran-3(2*H*)-one (47)

The crude product was prepared according to general procedure C starting from 4,6-difluorobenzofuran-3(2*H*)-one (**8**) and 4-butylbenzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane 1/cyclohexane 1) to yield a pure white solid (56%). m.p. 93–94°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 2H, J = 8.1 Hz, $H_{2',6'}$), 7.29 (d, 2H, J = 8.1 Hz, $H_{3',5'}$), 6.91 (s, 1H, -CH=), 6.88 (m, 1H, H_7), 6.63 (m, 1H, H_5), 2.68 (t, 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 1.65 (quint., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 1.39 (sext., 2H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$), 0.96 (t, 3H, J = 7.5 Hz, $PhCH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5, 170.1 (J = 13.0 Hz), 167.5 (J = 13.0 Hz), 160.8 (J = 16.2 Hz), 158.2 (J = 16.2 Hz), 146.4, 146.3, 131.9, 129.4, 129.2, 114.5, 100.0 (J_1 = 27.0 Hz, J_2 = 23.4 Hz), 97.5 (J_1 = 27.0 Hz, J_2 = 4.5 Hz). MS (ESI) *m/z* 315 $(M+H)^+$. Anal. Calcd for $C_{19}H_{16}F_2O_2$: C, 72.61, H, 5.14. Found: C, 72.86, H, 5.47.

(*Z*)-2-(2,4-difluorobenzylidene)-4-fluorobenzofuran-3(2*H*)-one (48)

The crude product was prepared according to general procedure C starting from 4-fluorobenzofuran-3(2*H*)-one (**9**) and 2,4-difluorobenzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane 1/cyclohexane 1) to yield a pure white solid (83%). m.p. 164–165°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (dt, 1H, J_1 = 8.6 Hz, J_2 = 6.5 Hz, $H_{6'}$), 7.65 (dt, 1H, J_1 = 8.3 Hz, J_2 = 5.5 Hz, H_6), 7.13 (d, 1H, J = 8.3 Hz, H_7), 7.11 (s, 1H, -CH=), 7.01 (m, 1H, $H_{5'}$), 6.90 (dt, 1H, J_1 = 8.7 Hz, J_2 = 2.6 Hz, $H_{3'}$), 6.88 (t, 1H, J = 8.3 Hz, H_5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 166.3 (J = 6.0 Hz), 164.4 (J_1 = 171.8 Hz, J_2 = 12.3 Hz), 161.9 (J_1 = 174.6 Hz, J_2 = 12.2 Hz), 160.1, 157.5, 146.9, 138.7 (J = 9.4 Hz), 133.2 (J = 9.5 Hz), 117.0 (J_1 = 12.0 Hz, J_2 = 4.1 Hz), 112.4 (J_1 = 21.2 Hz, J_2 = 2.9 Hz), 110.7 (J = 19.0 Hz), 109.0 (J = 4.1 Hz), 104.5 (J = 25.6 Hz), 103.9 (J = 5.7 Hz). MS (ESI) *m/z* 277 $(M+H)^+$. Anal. Calcd for $C_{15}H_7F_3O_2$: C, 65.23, H, 2.56. Found: C, 65.36, H, 2.57.

(*Z*)-2-(4-hydroxybenzylidene)-5-hydroxybenzofuran-3(2*H*)-one (49)

The crude product was prepared according to general procedure A starting from 5-hydroxybenzofuran-3(2*H*)-one (**4**) and 4-hydroxybenzaldehyde, and was recrystallized from acetonitrile to yield pure yellow crystals (17%). m.p. > 300°C (decomposition). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.21 (br s, 1H, OH), 9.76 (br s, 1H, OH), 7.85 (d, 2H, J = 7.8 Hz, $H_{2',6'}$), 7.38 (d, 1H, J = 8.5 Hz, H_6), 7.20 (d, 1H, J = 8.5 Hz, H_7), 7.01 (s, 1H, H_4), 6.89 (d, 2H, J = 7.8 Hz, $H_{3',5'}$), 6.83 (s, 1H, -CH=). ^{13}C NMR (100 MHz, d_6-DMSO) δ 183.4, 159.5, 158.8, 153.6, 145.5, 133.5, 125.2, 122.9, 121.5, 116.0, 113.7, 112.8, 107.4. MS (ESI) *m/z* 255 $(M+H)^+$, 277 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{10}O_4 \cdot ⅔H_2O$: C, 67.67, H, 4.29. Found: C, 67.56, H, 4.10.

(*Z*)-2-(4-cyclohexylbenzylidene)-4,6-dibenzyloxybenzofuran-3(2*H*)-one (50)

The crude product was prepared according to general procedure C starting from 4,6-dibenzyloxybenzofuran-3(2*H*)-one (**7**) and 4-cyclohexylbenzaldehyde, and was purified by column chromatography on silica gel (eluent: dichloromethane 2/cyclohexane 1) to yield a pure pale yellow solid (43%). m.p. 155–157°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8.2 Hz, $H_{2',6'}$), 7.32–7.50 (m, 10H, OCH_2Ph), 7.29 (d, 2H, J = 8.2 Hz, $H_{3',5'}$), 6.78 (s, 1H, -CH=), 6.46 (d, 1H, J = 1.6 Hz, H_7), 6.24 (d, J = 1.6 Hz, H_5), 5.28 (s, 2H, OCH_2Ph), 5.11 (s, 2H, OCH_2Ph), 2.53 (m, 1H, *c*-Hex), 1.89 (m, 4H, *c*-Hex), 1.78 (m, 1H, *c*-Hex), 1.43 (m, 4H, *c*-Hex), 1.30 (m, 1H, *c*-Hex). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 169.0, 167.8, 158.5, 150.0, 147.7, 136.2, 135.7, 131.4, 130.3, 129.0, 128.9, 128.7, 128.1, 127.8, 127.5, 127.0, 111.2, 106.1, 96.6, 90.7, 71.0, 70.9, 44.8, 34.4, 27.1, 26.3. MS (ESI) *m/z* 517 $(M+H)^+$. Anal. Calcd for $C_{35}H_{32}O_4 \cdot ⅓H_2O$: C, 80.46, H, 6.26. Found: C, 80.30, H, 6.67.

(*Z*)-2-((1-butyl-1*H*-indol-3-yl)methylene)-4,6-dihydroxybenzofuran-3(2*H*)-one (51)

The crude product was prepared according to general procedure B starting from 4,6-dihydroxybenzofuran-3(2*H*)-one (**3**) and 1-butyl-1*H*-indole-3-carbaldehyde, and was purified by column chromatography on silica gel (eluent: cyclohexane 2/ethyl acetate 1/acetic acid 0.005) to yield a pure red solid (65%). m.p. 248–249°C. ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.75 (s, 2H, OH), 8.13 (s, 1H, $H_{2'}$), 7.97 (d, 1H, J = 7.7 Hz, $H_{4'}$), 7.56 (d, 1H, J = 7.7 Hz, $H_{7'}$), 7.25 (t, 1H, J = 7.7 Hz, $H_{6'}$), 7.18 (t, 1H, J = 7.7 Hz, $H_{5'}$), 6.97 (s, 1H, -CH=), 6.27 (d, 1H, J = 1.2 Hz, H_7), 6.08 (d, 1H, J = 1.2 Hz, H_5), 4.28 (t, 2H, J = 7.2 Hz, $CH_2CH_2CH_2CH_3$), 1.76 (quint., 2H, J = 7.2 Hz, $CH_2CH_2CH_2CH_3$), 1.27 (sext., 2H, J = 7.2 Hz, $CH_2CH_2CH_2CH_3$), 0.88 (t, 3H, J = 7.2 Hz, $CH_2CH_2CH_2CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO) δ 180.3, 168.9, 168.5, 159.9, 147.3, 138.0, 134.9, 129.2, 124.5, 122.7, 121.1, 112.6, 109.4, 105.7, 104.2, 99.5, 92.5, 47.9, 33.9, 21.5, 15.6. Anal. Calcd for $C_{21}H_{19}NO_4$: C, 72.19, H, 5.48, N, 4.01. Found: C, 71.81, H, 5.79, N, 3.79.

(*Z*)-2-(3,4-dimethoxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (52)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and 3,4-dimethoxybenzaldehyde, and was recrystallized from methanol to yield pure pale yellow crystals (83%). m.p. 173–174°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 2H, $H_{2',6'}$), 6.91 (d, 1H, *J* = 8.1 Hz, $H_{5'}$), 6.73 (s, 1H, -CH=), 6.34 (s, 1H, H_7), 6.12 (s, 1H, H_5), 3.96 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.93 (s, 3H, OMe), 3.91 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 168.7, 159.3, 150.3, 148.9, 146.8, 125.5, 125.3, 113.5, 111.3, 111.1, 105.4, 93.9, 89.1, 56.2, 56.1, 55.9, 56.0. MS (ESI) *m/z* 343 $(M+H)^+$, 707 $(2M+Na)^+$.

(*Z*)-2-(2,4-dimethoxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (53)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and 2,4-dimethoxybenzaldehyde, and was recrystallized from methanol to yield pure pale yellow crystals (91%). m.p. 213–214°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 1H, *J* = 8.7 Hz, $H_{6'}$), 7.27 (s, 1H, -CH=), 6.57 (dd, 1H, *J* = 8.7 Hz, *J* = 2.3 Hz, $H_{5'}$), 6.45 (d, 1H, *J* = 2.3 Hz, $H_{3'}$), 6.36 (d, 1H, *J* = 1.7 Hz, H_7), 6.11 (d, 1H, *J* = 1.7 Hz, H_5), 3.94 (s, 3H, OMe), 3.90 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.86 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 168.5, 168.4, 162.2, 160.1, 159.2, 146.8, 132.8, 114.6, 105.6, 105.5, 105.3, 97.9, 93.7, 89.0, 56.1, 56.0, 55.5, 55.4. MS (ESI) *m/z* 343 $(M+H)^+$, 707 $(2M+Na)^+$.

(*Z*)-2-benzylidene-4,6-dimethoxybenzofuran-3(2*H*)-one (54)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and benzaldehyde, and was recrystallized from methanol to yield pure pale yellow crystals (61%). m.p. 156–157°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 1H, *J* = 7.3 Hz, $H_{2',6'}$), 7.39–7.48 (m, 3H, $H_{3',4',5'}$), 6.68 (s, 1H, -CH=), 6.66 (d, 1H, *J* = 1.5 Hz, H_7), 6.31 (d, 1H, *J* = 1.5 Hz, H_5), 3.91 (s, 3H, OMe), 3.89 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 168.9, 168.3, 158.9, 147.3, 132.1, 130.8, 129.3, 128.8, 109.3, 104.0, 94.3, 89.8, 56.4, 56.1. MS (ESI) *m/z* 283 $(M+H)^+$, 587 $(2M+H)^+$.

(*Z*)-2-(4-fluorobenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (55)

The crude product was prepared according to general procedure A starting from 4,6-dimethoxybenzofuran-3(2*H*)-one (**6**) and 4-fluorobenzaldehyde, and was recrystallized from methanol to yield pure pale yellow crystals (64%). m.p. 186–187°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (m, 2H, $H_{2',6'}$), 7.12 (m, 2H, $H_{3',5'}$), 6.74 (s, 1H, -CH=), 6.40 (d, 1H, *J* = 1.8 Hz, H_7), 6.15 (d, 1H, *J* = 1.8 Hz, H_5), 3.97 (s, 3H, OMe), 3.93 (s, 3H, OMe). ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 168.2, 163.2 (d, *J* = 251.4 Hz), 159.6, 147.7, 133.2 (d, *J* = 8.2 Hz), 129.1, 116.2 (d, *J* = 21.7 Hz), 109.8, 105.4, 94.3, 89.4, 56.4, 56.4. MS (ESI) *m/z* 301 $(M+H)^+$, 623 $(2M+Na)^+$.

Molecular Modeling

Docking studies used the reported X-ray crystallographic structure of HCV genotype 1b RdRp complexed with a known inhibitor binding to Thumb Pocket I of the enzyme (PDB ID: 2dxs).29 The protein structure was extracted and water molecules were deleted. The complexed ligand was used to define the binding site for docking. The genetic algorithms of GOLD and Autodock4 docking softwares performed flexible docking with small molecules while keeping the protein structure rigid. All compounds were built and their energies minimized with Sybyl, using a Conjugate Gradient method and MMFF94 force field and charges. For each compound, 20 poses were generated by GOLD and ranked according to the Goldscore scoring function, while 100 poses were generated by Autodock and clustered according to their spatial proximity and calculated free energy of binding. Molecular surfaces were generated with MOLCAD and the docking solutions were analyzed with Sybyl.

Biology

Expression and purification of recombinant HCV NS5BΔ21

RdRp (NS5B protein) from the HCV J4 genotype 1b reference strain, truncated of its 21 C-terminal amino acids to ensure solubility (NS5BΔ21) and carrying a hexahistidine tag (His-Tag) at its N-terminus, was expressed in *Escherichia coli* C41(DE3) and purified. Chromatography was performed on Ni-NTA columns (Qiagen, Valencia, California). The columns were washed with a buffer containing 50 mM NaH_2 PO_4 (pH 8.0), 500 mM NaCl, and 20 mM imidazole. The bound protein was eluted in 1-mL fractions with a buffer containing 50 mM NaH_2 PO_4 (pH 8.0), 500 mM NaCl, and 250 mM imidazole. NS5BΔ21-enriched fractions were selected with a Bradford colorimetric assay, and NS5BΔ21 purity was determined by SDS-PAGE analysis with Coomassie staining. Purified NS5BΔ21 fractions were pooled and dialyzed against a buffer containing 5 mM Tris (pH 7.5), 0.2 M sodium acetate, 1 mM DTT, 1 mM EDTA, and 10% glycerol. NS5BΔ21 purity was >98%.

HCV-NS5BΔ21 polymerase assay

An enzyme assay was used to measure inhibition of HCV-NS5BΔ21 polymerase activity. The cell-free HCV-NS5BΔ21 polymerase assay is based on the amount of double-stranded RNA synthesized in the presence of HCV-NS5BΔ21, a homopolymeric RNA template (Poly U, GE Healthcare, Chalfont St. Giles, UK) and ATP, measured with an intercalating agent (SYBR® Green, Applied Biosystems), as previously described.30 The Quick Site-Directed Mutagenesis kit (Stratagene, La Jolla, California) was used to assess the effect of amino acid substitutions conferring resistance to other specific HCV RdRp inhibitors. The tested substitutions included M423T, C316Y, H95Q, and P495L. The constructs were verified by DNA sequencing and the corresponding proteins were purified as described above.

Cytotoxicity assay

Exponentially growing HuH7 and HEK293 cells were trypsinized and plated (2000 and 1000 cells per well, respectively) in 96-well microplates and allowed to attach for 96 hours at 37°C under 5% CO_2 . A 200-μL aliquot of drug solution, diluted in medium with 1% DMSO (final concentration) was added and the cells were incubated for 72 hours at 37°C under 5% CO_2 . Cytotoxicity was evaluated with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay (MTT assay).

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Figure 1

Two examples of known Thumb Pocket I inhibitors (top); general structure of the aurone backbone (bottom left); and structure of naturally occurring aureusidin (compound **1** , bottom right).

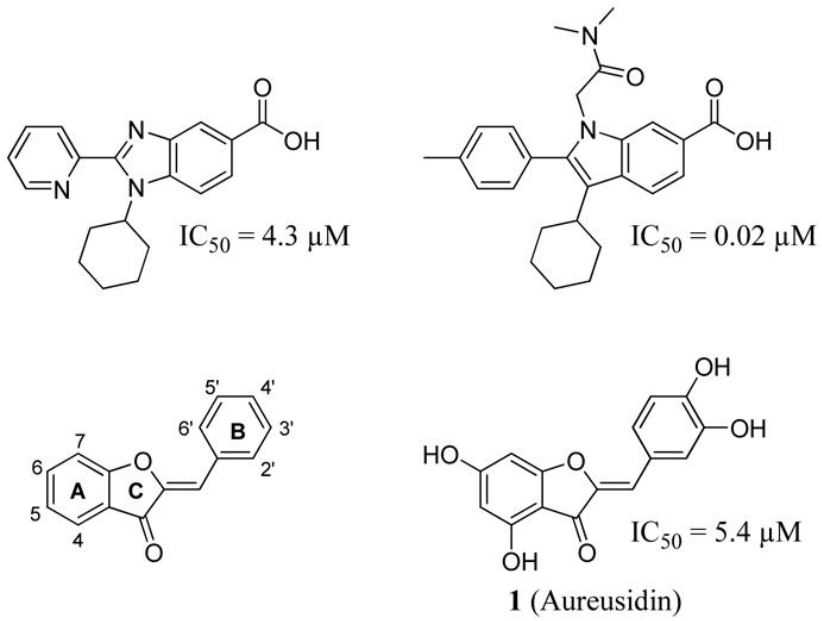


Figure 2

Structure of HCV RdRp with its three subdomains (Fingers in red, Palm in blue and Thumb in green) and its four allosteric binding sites for known non-nucleoside inhibitors. Key substitution sites of adjacent amino acids are indicated in bold.

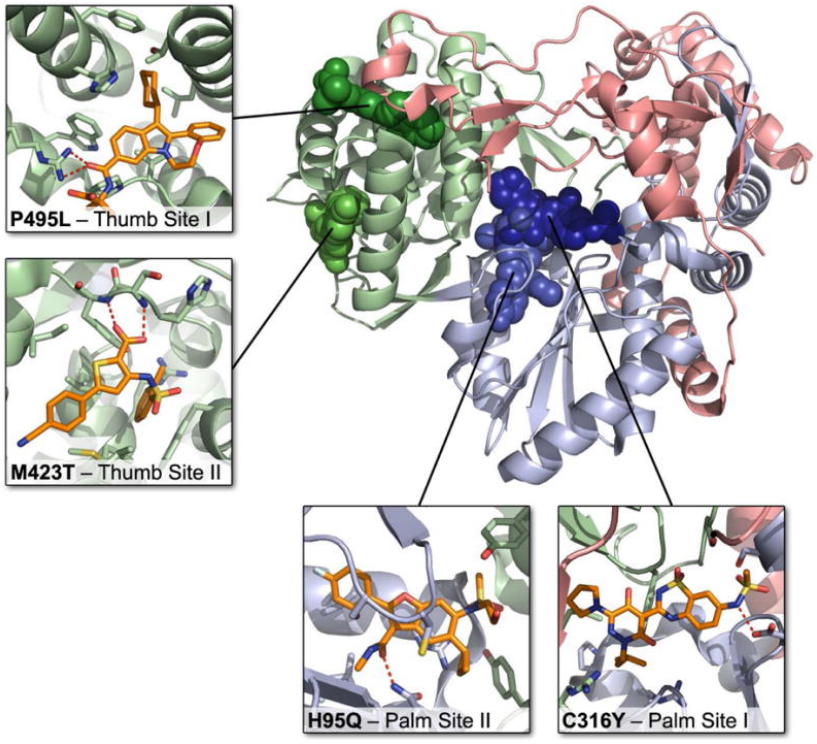


Figure 3

Fold increase in IC_{50} relative to wild-type NS5BΔ21 obtained with aurone **11** and P495L, H95Q, C316Y and M423T mutants; these mutations are known to reduce susceptibility to Thumb Pocket I, Palm Pocket II, Palm Pocket I and Thumb Pocket II inhibitors, respectively.

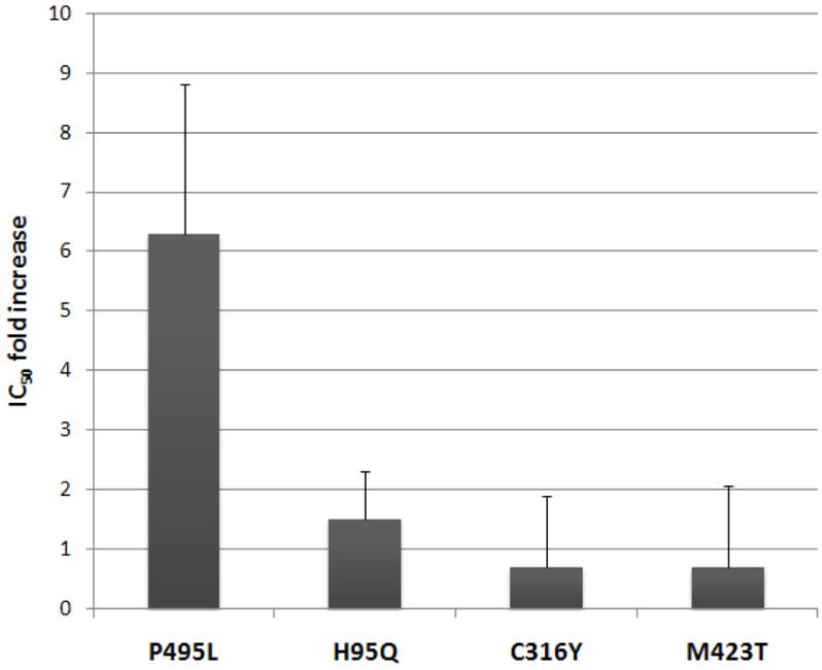
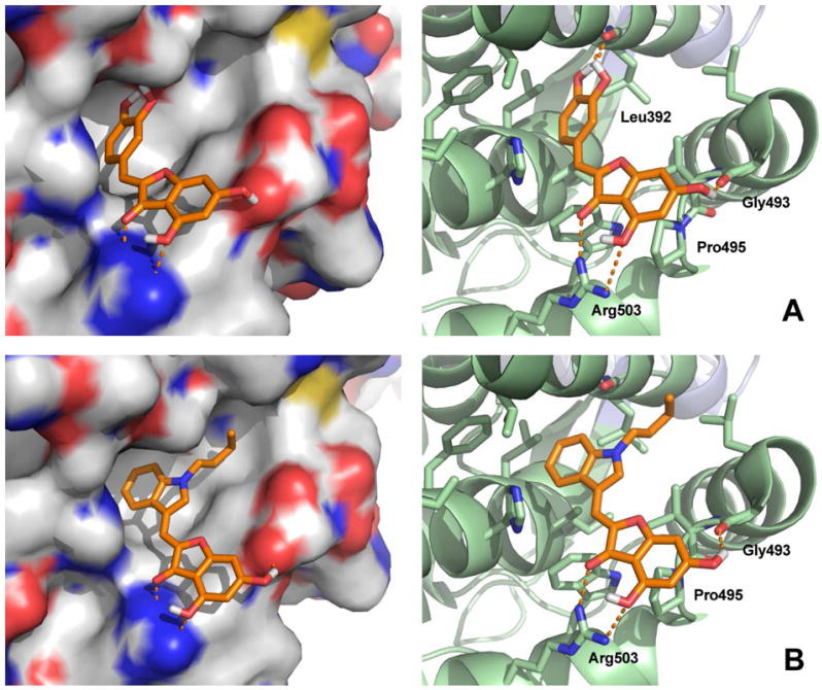


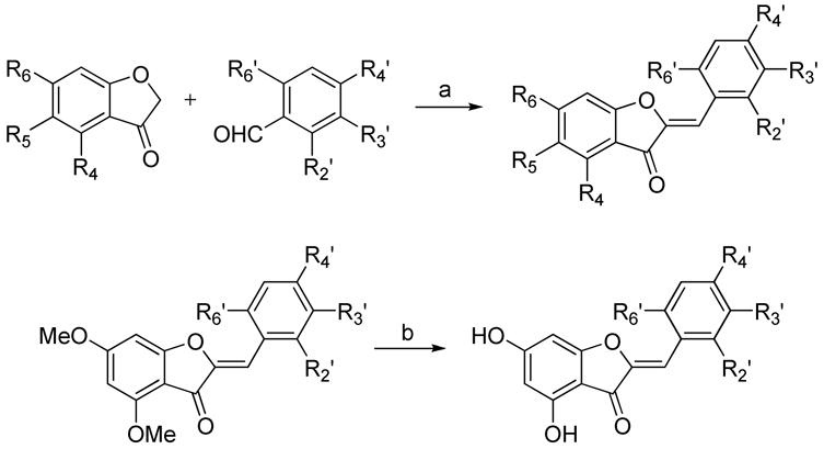
Figure 4

Surface and ribbon views of predicted binding modes in RdRp Thumb Pocket I of a reported X-ray structure (PDB id: 2dxs) from docking studies with Autodock. (A) For compound **1** , involved in five hydrogen bonds: two with Arginine 503 (1.9 Å and 2.5 Å), one with Glycine 493 (1.8 Å) and two with Leucine 392 (2.0 Å and 2.0 Å). (B) For compound **51** , involved in three hydrogen bonds: two with Arginine 503 (1.8 Å and 2.0 Å) and one with Glycine 493 (2.2 Å). The pictures were built with Pymol software.



Scheme 1

Synthesis of aurone derivatives, starting from building blocks **2 – 9** [a a] Reagents and conditions: (a) for Procedure A: KOH/MeOH, 60°C, 1 – 18 h; for Procedure B: KOH/EtOH, 80°C, 2 – 5 h; for Procedure C: Al_2O_3, CH_2Cl_2, rt, 16 h. (b) BBr_3, CH_2Cl_2, 0°C to rt, 24 – 72 h.



Scheme 2

Synthesis of benzofuran-3(2*H*)-one derivatives **2 – 9** as the key starting blocks [a a] Reagents and conditions: (a) $ClCH_2CN$, HCl, $ZnCl_2$, Et_2O, then HCl, H_2O, 100°C, 1 h; (b) MeONa, MeOH, 60°C, 1.5 h; (c) trimethylphenylammonium tribromide, THF, rt, 6 h; (d) KOH, MeOH, 60°C, 3 h; (e) Me_2SO_4, K_2CO_3, dimethoxyethane, 80°C, 3 h; (f) BnBr, K_2CO_3, dimethoxyethane, rt, 3 days; (g) chloroacetic acid, NaOH, H_2O, 90°C, 18 h; (h) $SOCl_2$, PhMe, 110°C, 2 h, then $AlCl_3$, CH_2Cl_2, 40°C, 18 h; (i) $CuBr_2$, EtOAc, $CHCl_3$, 60°C, 18 h; (j) K_2CO_3, DMF, 0°C, 1.5 h.

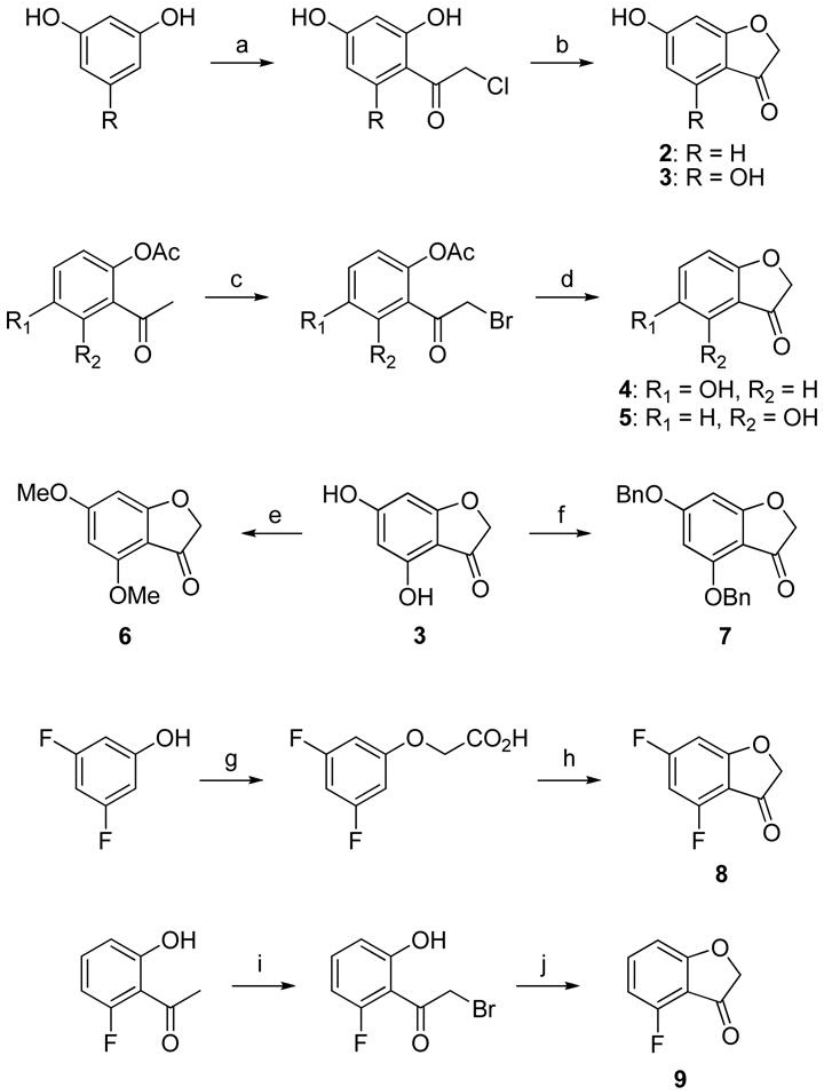


Table 1

Initial array of NS5B inhibition by aureusidin analogues (compounds **10 – 20**).

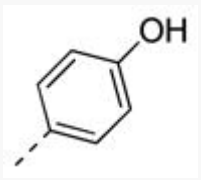
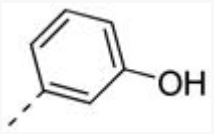
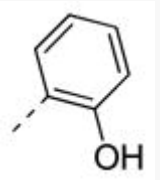
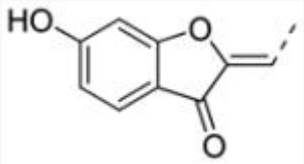
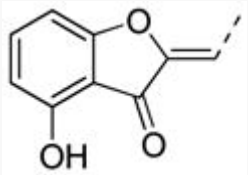
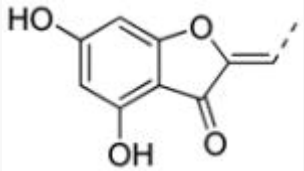
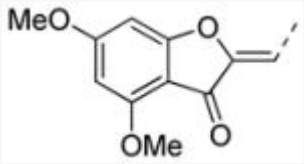
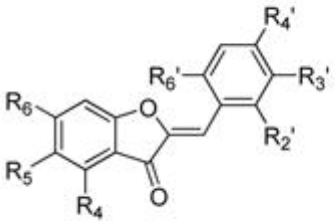
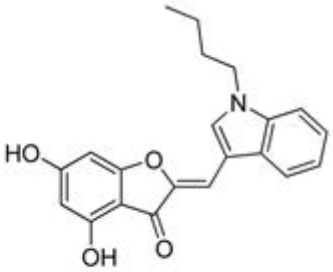
									
	Cd	**Inh. (20 μM, %)**	**IC_{50} (μM)**	**Cd**	**Inh. (20 μM, %)**	**IC_{50} (μM)**	**Cd**	**Inh. (20 μM, %)**	**IC_{50} (μM)**
	**10**	35.3	n.d.	**14**	10.3	n.d.	**17**	18.4	n.d.
	**11**	60.3	5.9	**15**	37.7	n.d.	**18**	56.0	11.1
	**12**	60.9	9.7				**19**	33.6	n.d.
	**13**	37.6	n.d.	**16**	11.7	n.d.	**20**	0.7	n.d.

Table 2

Inhibition activity of compounds **1** and **21 – 51** .



1, 21 - 50



51

Compd	R_4	R_5	R_6	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{6'}$	Inhibition (20 μM, %)	IC_{50} (μM)
1	OH	H	OH	H	OH	OH	H	85.0	5.4
21	OH	H	H	H	H	H	H	57.8	14.6
22	OH	H	H	OH	H	OH	H	94.1	2.6
23	OH	H	H	Me	H	Me	H	64.2	5.8
24	OH	H	H	OMe	H	OMe	H	75.6	9.7
25	OH	H	H	OMe	H	H	OMe	47.2	n.d.
26	OH	H	H	OMe	H	OMe	OMe	51.8	n.d.
27	OH	H	H	H	H	Et	H	46.5	n.d.
28	OH	H	H	H	H	*i* -Pr	H	29.9	n.d.
29	OH	H	H	H	H	*n* -Bu	H	42.7	n.d.
30	OH	H	H	H	H	*t* -Bu	H	60.8	n.d.
31	OH	H	H	H	H	*N* -Me-Ppz	H	94.4	3.8
32	OH	H	H	F	H	H	H	58.5	n.d.
33	OH	H	OH	H	H	H	H	59.1	15.1
34	OH	H	OH	OH	H	OH	H	80.8	5.8
35	OH	H	OH	OMe	OMe	H	H	74.5	n.d.
36	OH	H	OH	OMe	OMe	OMe	H	50.2	n.d.
37	OH	H	OH	H	H	Et	H	79.7	3.6
38	OH	H	OH	H	H	*i* -Pr	H	78.3	3.1
39	OH	H	OH	H	H	*n* -Bu	H	93.4	2.5
40	OH	H	OH	H	H	*c* -Hex	H	91.8	2.3
41	OH	H	OH	H	H	morpholinyl	H	66.6	11.4
42	OH	H	OH	H	H	F	H	80.5	11.2
43	OMe	H	OMe	Me	H	Me	H	14.1	n.d.
44	OMe	H	OMe	H	H	*n* -Bu	H	3.3	n.d.
45	OMe	H	OMe	H	H	*c* -Hex	H	2.0	n.d.
46	H	H	H	H	H	*n* -Bu	H	-33.8	n.d.
47	F	H	F	H	H	*n* -Bu	H	-33.0	n.d.
48	F	H	H	F	H	F	H	10.6	n.d.
49	H	OH	H	H	H	OH	H	46.6	n.d.
50	OBn	H	OBn	H	H	*c* -Hex	H	3.6	n.d.

51		95.1	2.2