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Original article

B-ring modified aurones as promising allosteric inhibitors of hepatitis C virus RNA-dependent RNA polymerase

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ABSTRACT

Following our recent report showing the potential of naturally occurring aurones (2-benzylidenebenzofuran-3(2*H*)-ones) as anti-hepatitis C virus (HCV) agents, efforts were continued in order to refine the structural requirements for the inhibitory effect on HCV RNA-dependent RNA polymerase (RdRp). In this study, we targeted the B-ring moiety of aurones with the aim to improve structural features associated with higher inhibition of the targeted polymerase. In vitro evaluation of the RdRp inhibitory activity of the 37 newly synthesized compounds pointed out that the replacement of the B-ring with an *N*-substituted indole moiety induced the highest inhibitory effect. Of these, compounds **31**, **40** and **41** were found to be the most active (IC_{50} = 2.3–2.4 μM). Docking experiments performed with the most active compounds revealed that the allosteric thumb pocket I of RdRp is the binding pocket for aurone analogues.

1. Introduction

Belonging to the *Hepacivirus* genus of the *Flaviviridae* family, hepatitis C virus (HCV) is responsible for a chronic infection of global health concern since it affects more than 170 million individuals – approximately 3% of the world population. The acute infection is often asymptomatic. Nevertheless, HCV infection can become chronic and chronic hepatitis C can progress to cirrhosis, end-stage liver disease, hepatocellular carcinoma and death [1,2]. To date, there is no prophylactic anti-HCV vaccine. Until the recent approval of two protease inhibitors, the standard treatment was the use of a combination of pegylated interferon α (peg-IFN) and ribavirin (RBV) [3,4]. However this non-specific therapy has significant side-effects and was of limited efficiency since only a 40–50% sustained virologic response rate (SVR) was reached in patients infected with HCV genotype 1, the most prevalent genotype in Europe and North America [5,6]. The approval in 2011 of the first-generation HCV NS3 protease inhibitors telaprevir and boceprevir in combination with pegylated interferon and ribavirin improved SVR rates in patients with HCV genotype 1 infection and eventually shortened treatment duration [7]. However, these therapies were poorly tolerated and resistance to the protease inhibitor emerged as an issue in patients who failed on therapy. Therefore, the need for new direct-acting antivirals (DAAs) with a high therapeutic index is of importance [8,9].

Increased understanding of the HCV life cycle has resulted in the discovery of several viral targets for specific antiviral therapy [10]. HCV is a small, positive-sense, single-stranded RNA virus with a 9.6 kb-genome that is translated into a polypeptide of approximately 3000 amino acids. This poly-protein can be cleaved by a combination of host and viral proteases into ten viral proteins – structural (core, E1, E2 and p7) and non-structural (NS2, NS3, NS4A NS4B, NS5A and NS5B) ones [11]. Amongst HCV proteins investigated as targets for anti-HCV therapeutic development, non-structural proteins NS3 and NS5B seem to be the most promising candidates [12,13].

The NS5B RNA-dependent RNA polymerase (RdRp) is a particularly attractive target because it is the key enzyme that catalyzes viral replication, with no close mammalian counterpart. This 65 kDa enzyme shares the common structural features of other

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0223-5234/

viral polymerases, shaped as a 'right hand' with characteristic thumb, finger and palm domains [14–17]. In addition to the catalytic site, at least four allosteric binding sites have been identified so far, including 'thumb' pockets I and II and 'palm' pockets I and II/III [18–21]. A number of HCV RdRp inhibitors have been identified, including nucleoside/nucleotide analogues that target the active site, and non-nucleoside allosteric inhibitors [22,23]. Both types of drugs have been shown to be important in new interferon-free, oral drug combination strategies against HCV.

Recently, we showed that naturally occurring aurones (2-benzylidenebenzofuran-3(2*H*)-ones, Fig. 1) – small molecules belonging to the flavonoid family – have potent anti-HCV properties *in vitro* [24]. In this study, it was proven that aurones, represented by the naturally occurring aureusidin (3′,4,4′,6-tetrahydroxyaurone, **1**, Fig. 1), potently inhibit HCV RdRp, with an IC_{50} of 5.2 μM. Moreover, the binding site of this family of compounds was unambiguously identified to be thumb pocket I using site-directed mutagenesis experiments confirmed by molecular modeling. This first structure–activity relationship study allowed us to partially identify the key positions and substituents that are important for the inhibitory activity of HCV RdRp. Among others, the 4,6-dihydroxybenzofuran-3(2*H*)-one moiety was found to be crucial for the inhibition activity. Based on these results, the aim of the present investigation was to maintain the 4,6-dihydroxybenzofuran-3(2*H*)-one moiety and to determine the aryl type that could replace the B-ring of aurones in order to enhance the inhibitory activity on HCV RdRp. The aryl ring replacing the B-phenyl of aurones was chosen among substituted phenyls, bicyclic, aryl fused moieties or small oxygen- or nitrogen-containing heterocycles. Finally, the investigation of aurones as potential anti-HCV drugs was motivated by the very low toxicity of naturally occurring aurones and derivatives. Even at high concentration (>400 μM), aurones were not cytotoxic (MTT assay on HuH7 and HEK293) [24]. Furthermore, one 4,6-dihydroxyaurone derivative was evaluated in a previous report through *in vivo* oral and dermal toxicity assays and no toxicity was observed in standard concentrations [25].

2. Results and discussion

2.1. Chemistry

To synthesize the aurones of interest, we followed a previously reported general and convenient synthetic pathway involving an aldol condensation between arylaldehydes and 4,6-dihydroxybenzofuran-3(2*H*)-one under basic conditions (Scheme 1) [25,26]. The synthesis of aurone derivatives **4**–**38** was accomplished according to Scheme 1.

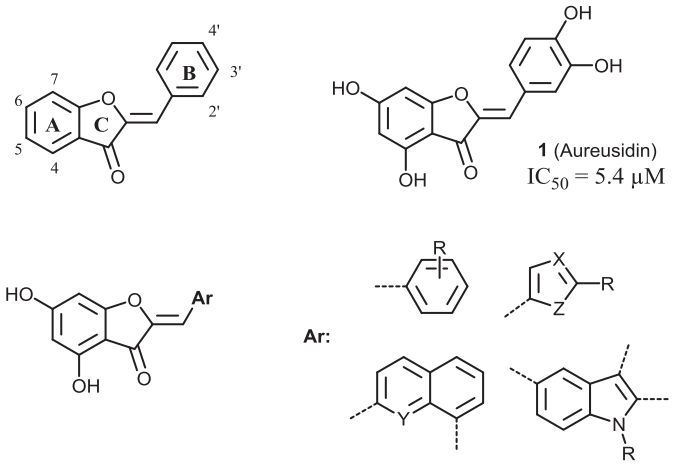


Fig. 1. General structure of the aurone backbone (top left), structure and activity of the naturally occurring aureusidin **1** and structures of aurone derivatives investigated in this study (the dashed line indicate the site of linkage).

The prerequisite 4,6-dihydroxybenzofuran-3(2*H*)-one **3** was first synthesized in two steps. Reaction of phloroglucinol with chloroacetonitrile in HCl/Et_2O according to Houben–Hoesch reaction afforded the chloroacetophenone **2** which was directly cyclised in acidic media to afford the 4,6-dihydroxybenzofuran-3(2*H*)-one **3** with 84% yield for two steps.

Most of aromatic aldehydes used in the aldol condensation were commercially available except 2,4-dibutoxybenzaldehyde, employed for the synthesis of compound **7** (Table 1), and indole derivatives, used for the synthesis of compounds **20**–**38** (Table 2). The 2,4-dibutoxybenzaldehyde was synthesized starting from 2,4-dihydroxybenzaldehyde and using 1-bromobutane and potassium carbonate in DMF while *N*-substituted indolecarboxaldehydes were obtained by alkylation of the corresponding indolecarboxaldehyde with alkyl halides in the presence of sodium hydride 60% (41–99%, Scheme 2) [27].

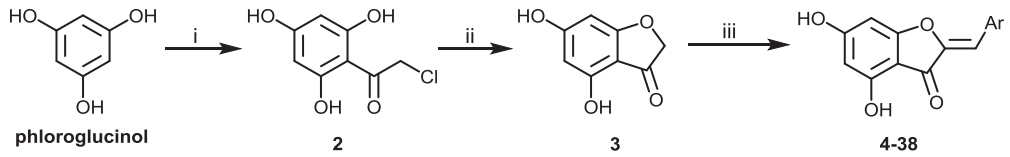
Finally, aurone derivatives bearing a hydroxyl group at the *N*-substituted indole moiety (**40**, **41** and **42**) were obtained by demethylation of the corresponding methoxy derivatives (**30**, **36** and **38**), using boron tribromide according to Scheme 3.

2.2. In vitro evaluation of HCV-NS5BΔ21 polymerase activity inhibition

An enzyme assay was used to assess the inhibition of the polymerase activity of a purified RdRp (NS5B protein), deleted of its 21 C-terminal amino acids in order to ensure solubility (HCV-NS5BΔ21). The J4 genotype 1b reference strand was used. 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 20 μM concentration of aurone followed by the measurement of IC_{50} of those that showed higher activity at 20 μM.

Our previous study pointed out some pharmacophoric elements that were crucial for the inhibitory activity of aurones on HCV RdRp: (a) a hydroxyl group at position 4 or hydroxyl groups at positions 4 and 6 and (b) a 2′,4′- or 3′,4′-dihydroxylated B-ring or a hydrophobic and bulky substituent or alternative core at the B-ring [24]. In order to refine this first study, new aurones were then synthesized. Firstly, to expand the structure–activity relationship and to improve our knowledge concerning the binding mode of this class of compounds with their target, new synthesized aurone derivatives were evaluated in which the B-ring was replaced by: (a) phenyl ring bearing weak polar and more or less bulky substituents (**4**–**8**, Table 1), (b) bis-aryl or fused aryl rings (**9**–**12**). Most of these compounds presented a fairly good activity, in the micromolar range, except for compounds **4** and **11** whose IC_{50} values were not determined.

During our previous study, it was shown that aurone derivatives bearing an indolyl moiety were promising NS5B inhibitors (compound **19**, IC_{50} = 2.2 μM, Table 2) [24]. We then naturally proceeded in our study synthesizing both new heterocyclic aurones and *N*-functionalized indoles. The B-ring was subsequently exchanged with quinoline, benzimidazole, imidazole, pyrrole and furane cores (**13**–**18**, Table 1), but unfortunately none of these heterocycles gave a noticeable gain of activity. We can therefore conclude that the inhibition of HCV-NS5BΔ21 polymerase activity revealed that B-ring can be replaced by a variety of aryls but no smaller than phenyl, indicating that the B-ring of aurones might interact with a large pocket within the binding site.



Scheme 1. General pathway for the synthesis of aurone derivatives. Reagents and conditions: (i) $ClCH_2CN$, HCl, $ZnCl_2$, Et_2O, 0 °C; (ii) H_2O, 100 °C, 5 h, 84% (for 2 steps); (iii) ArCHO, KOH 50% in H_2O, EtOH, reflux, 2–15 h.

Contrariwise, all the new B-ring indole derivatives synthesized (**20**–**38** and **40**–**42**, Table 2) were found as single-digit micromolar inhibitors. We first began our study evaluating the influence of the attachment position of the indole on the benzofuranone moiety. Comparison of the biological *in vitro* activity of compounds **19**–**21** did not point out a major impact of this parameter even if compound **20** seems to be slightly less active. Interestingly, *N*-methyl compound **22** reached a similar IC_{50} value than *N*-butyl **21** (IC_{50} = 3.10 μM *vs.* 3.00 μM respectively), while a similar comparison between indol-3-ylmethylene analogues showed a two-fold drop of activity in the case of an *N*-methyl substitution (IC_{50} = 6 μM for **23** *vs.* 2.2 μM for **19**). Furthermore, we assessed the effect of the *N*-substituent on the 2-[(indol-3-yl)methylene]-4,6-dihydroxybenzofuran-3(2*H*)-one moiety (compounds **24**–**33** and **40**). All substituents were globally well tolerated and the corresponding aurones mainly led to inhibition levels in the same range (IC_{50} = 2.4–3.3 μM), except more polar substituents (**26**). These results tend to confirm the interaction pattern of aurones with NS5B previously described for compound **19** [24]. Indeed, the binding pocket appears to be quite large, since even bulky substituents can be accommodated (*e.g.* IC_{50} = 2.7 μM for *N*-phenylpropyl compound **33**), and rather hydrophobic, as previously stated (the B-ring was expected to be surrounded by residues Leu392, Val494, Ile424 and Leu425). Finally, diversely substituted *N*-butylindole cores were evaluated (compounds **34**–**38** and **41**–**42**). Once again, these modifications were well tolerated except for the 5-hydroxy substitution of **42** which induced a great loss of activity. Nonetheless the same polar substituent at position 7 of the indole is not deleterious (IC_{50} = 2.3 μM for **41**).

Altogether, through these results, the attachment position to the benzofuranone core and the nature of indole *N*-, 5- or 7-substituent revealed having far less influence. The majority of indole analogues evaluated in this study were found in a narrow range of inhibition activity (between 2.0 and 3.5 μM for at least 12 compounds). This high level of tolerance confirmed that general hydrophobic, Van der Waals interactions play a prominent role at the B-ring of aurones; therefore it might be difficult to enhance the efficiency of aurones as NS5B inhibitors by simply further modulating this position.

2.3. Interaction of aurone derivatives with the allosteric thumb pocket I of NS5B

Using site-directed mutagenesis studies, we unambiguously identified the thumb pocket I as the binding site of aurones [24]. This information was used to dock representatives of studied aurones into the binding pocket using Autodock tool (Fig. 2). The docking results revealed the presence of two hot spot residues, Arg503 and Gly493, interacting with 4- and 6-hydroxy groups of aurones, respectively, as already described for compound **19**. While the general geometry in the binding site remains very similar for each analogue, the benzofuranone cores of indol-2-ylmethylene derivatives such as compound **21** are inverted, and subsequently build hydrogen bonds with Arg503 and Gly493 with 6-hydroxy and 4-hydroxy substituents respectively. Interestingly, compound **41**, bearing a hydroxyl group at C-7 of the indole ring establishes an extra interaction with a leucine residue (Leu392). The latter interaction may explain the higher activity of **41** compared to its closed analogue **42** bearing a hydroxyl group at C-5 of the indole ring. Overall, the close IC_{50} values obtained for these compounds is also seen in terms of geometry and predicted free energies of binding (between −7.90 and −6.90 kcal/mol for almost all compounds).

3. Conclusion

In conclusion, we report herein on the investigation of compounds derived from the naturally occurring aurones as HCV RdRp inhibitors. Our main objective was the pharmacomodulation of the B-ring of aurones in order to identify the optimal aryl ring. The first finding highlighted the importance of the aryl size with the indole ring being the most favorable among diverse aryl rings tested. The majority of the aurones bearing indole rings exerted a remarkable inhibition activity on HCV RdRp with IC_{50} values in the one digit micromolar order. Modeling studies have suggested the ability of the aurone structures to establish key interactions with three residues surrounding the thumb pocket I of HCV RdRp. The promising activity of aurones combined with the very low toxicity of this class of compounds may place them at the center of future anti-HCV drug research.

4. Experimental

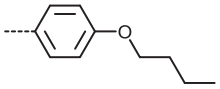
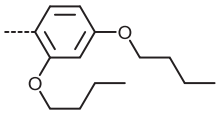
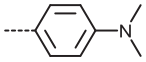
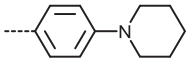
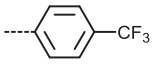
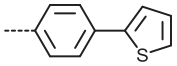
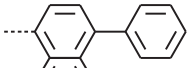
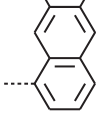
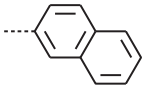
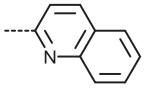
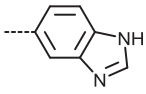
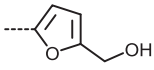
4.1. Chemistry

Commercially available reagents and solvents were used without further purification. Reactions were monitored by thin-layer chromatography (plates coated with silica gel 60 F_{254} from Merck). Silica gel 60 (70–230 mesh from Macherey–Nagel) was used for flash chromatography. Melting points were measured on a Fisher micromelting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded at room temperature in deuterated solvents on a Brüker AC-400 instrument (400 MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS as internal standard or relative to the solvent [^1H: δ(DMSO) = 2.50 ppm, $\delta(CDCl_3)$ = 7.24 ppm; ^{13}C: δ(DMSO) = 39.51 ppm, $\delta(CDCl_3)$ = 77.23 ppm]. 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. Accurate mass measurements (HRMS) were carried out on a TOF spectrometer, realized by the mass spectrometry analysis facilities of Orleans University (France).

4.1.1. 4,6-Dihydroxybenzofuran-3(2H)-one (**3**)

To a solution of phloroglucinol (15.0 g, 119 mmol) in diethyl ether (250 mL) at 0 °C were added chloroacetonitrile (7.5 mL, 119 mmol) and dried zinc chloride (1.62 g, 11.9 mmol). The mixture was allowed to react with gaseous hydrochloric acid at 0 °C for 1 h. The resulting suspension was stirred at 0 °C for 1.5 h, and was allowed to react again with gaseous HCl for 30 min. The precipitate was filtered, washed with ether, dried and suspended in water (200 mL). The solution was refluxed for 5 h. After cooling, the

Table 1
Structures and inhibition activity of synthesized aurone derivatives. The dashed bound indicates the site of attachment to the methylenebenzofuranone moiety.

Compd	Ar	Yield (%)	Inhibition (20 μM, %)	IC_{50} (μM)
4		63s	81 ± 3	n.d.
5		68	90 ± 3	4 ± 2
6		68	90 ± 1	5 ± 3
7		69	93 ± 1	5.5 ± 0.9
8		71	96.00 ± 0.04	5.4 ± 0.4
9		66	97.0 ± 0.1	4 ± 2
10		78	93 ± 1	4 ± 1
11		81	45 ± 27	n.d.
12		88	95 ± 1	3.4 ± 0.7
13		81	42.0 ± 0.6	n.d.
14		75	81 ± 2	10 ± 3
15		97	39 ± 1	>16
16		75	55 ± 2	>16
17		90	27 ± 7	>16
18		53	33 ± 1	>16

n.d.: not determined.

Table 2
Structures and inhibition activity of synthesized aurone indolic derivatives. The dashed bound indicates the site of attachment to the methylenebenzofuranone moiety.

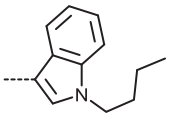
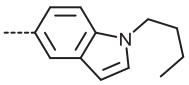
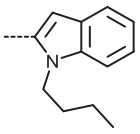
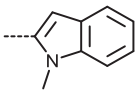
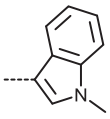
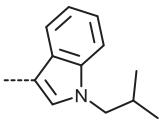
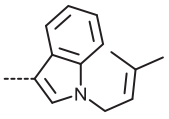
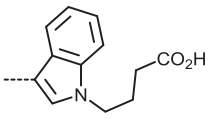
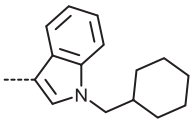
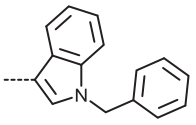
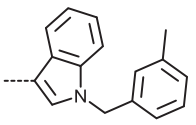
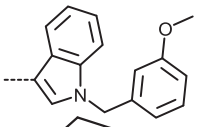
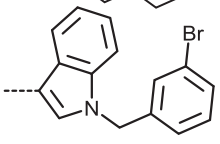
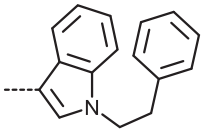
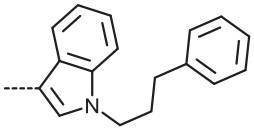
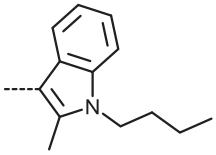
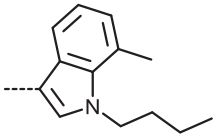
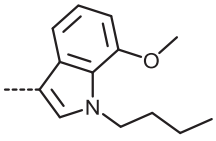
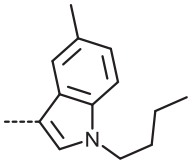
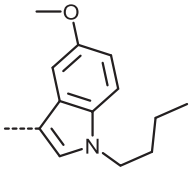
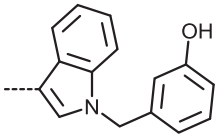
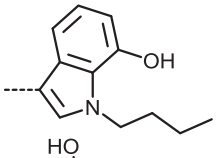
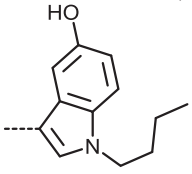
Compd	Ar	Yield (%)	Inhibition (20 μM, %)	IC_{50} (μM)
19[a]		65	95 ± 1	2.2 ± 0.2
20		72	94.3 ± 0.6	n.d.
21		54	98.4 ± 0.5	3.00 ± 0.04
22		66	97.8 ± 0.0	3.10 ± 0.08
23		78	90.0 ± 0.9	6 ± 1
24		81	80.70 ± 0.07	8 ± 3
25		80	94.7 ± 0.9	3.0 ± 0.1
26		56	70 ± 2	n.d.
27		87	93 ± 1	3.3 ± 0.4
28		73	96.2 ± 0.5	2.6 ± 0.6
29		54	95.6 ± 0.2	2.6 ± 0.2
30		72	95.4 ± 0.8	2.70 ± 0.05
31		62	96.0 ± 0.7	2.40 ± 0.07

Table 2 (*continued*)

Compd	Ar	Yield (%)	Inhibition (20 μM, %)	IC_{50} (μM)
32		46	93.9 ± 0.1	3.2 ± 0.5
33		65	93.0 ± 0.6	2.7 ± 0.3
34		56	91.6 ± 0.5	n.d.
35		61	92.5 ± 0.4	n.d.
36		78	93.1 ± 0.2	n.d.
37		81	96 ± 1	n.d.
38		85	93.8 ± 0.1	n.d.
40		93[b]	99.3 ± 0.2	2.4 ± 0.2
41		95[b]	98.9 ± 0.2	2.3 ± 0.2
42		96[b]	53.5 ± 0.3	n.d.

n.d.: not determined.

[a] Compound already published [24].

[b] These yields correspond to the last step of the synthesis (hydrolysis in presence of BBr_3).

crystals were filtered, washed with cold water and dried to afford **4** (16.6 g, 100 mmol, 84%) as orange crystals, which were analytically pure and used without further purification. m.p. = 257–259 °C [29]; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.61 (s, 1H), 10.58 (s, 1H), 5.91 (s, 2H), 4.55 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 193.9 (C), 175.6 (C), 167.5 (C), 157.4 (C), 102.6 (C), 96.1 (CH), 90.1 (CH), 74.8 (CH_2).

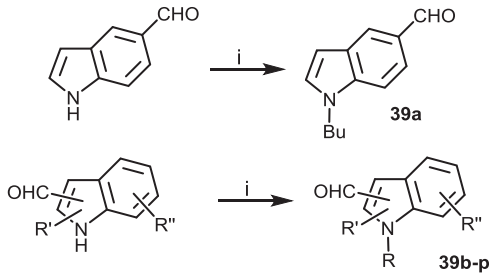
*4.1.2. General procedure A for the synthesis of compounds (**4**–**18** and **20**–**38**)*

To a solution of **3** in ethanol (3 mL/mmol) were added an aqueous solution of potassium hydroxide (50%, 5 mL/mmol) and a benzaldehyde derivative (1.0–3.5 equiv.). The solution was refluxed until TLC showed complete disappearance of the starting material (2–5 h). After cooling, ethanol was removed under reduced pressure, then the residue was diluted into distilled water (50 mL/mmol) and an aqueous solution of hydrochloric acid (10%) was added to adjust the pH to 2–3. The mixture was then extracted with ethyl acetate or dichloromethane. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered off and concentrated under reduced pressure to afford the corresponding crude (*Z*)-2-benzylidenebenzofuran-3(2*H*)-one derivative.

*4.1.2.1. (Z)-2-(4-butoxybenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**4**).* The crude product was prepared according to procedure **A** starting from **3** (166 mg, 1.00 mmol) and 4-butoxybenzaldehyde (356 mg, 2.0 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:2), the pure product (205 mg, 0.63 mmol, 63%) was obtained as a yellow powder. m.p. = 218–219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.88 (bs, 2H), 7.84 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.8 Hz, 2H), 6.58 (s, 1H), 6.21 (d, J = 1.6 Hz, 1H), 6.07 (d, J = 1.6 Hz, 1H), 4.02 (t, J = 6.4 Hz, 2H), 1.76–1.66 (m, 2H), 1.50–1.38 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 179.0 (C), 167.6 (C), 167.1 (C), 159.5 (C), 158.2 (C), 146.4 (C), 132.5 (2 × CH), 124.7 (C), 114.9 (2 × CH), 108.5 (CH), 102.7 (C), 97.7 (CH), 90.5 (CH), 67.3 (CH_2), 30.6 (CH_2), 18.7 (CH_2), 13.7 (CH_3); LRMS (ESI+) *m/z* (%) 327 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{19}H_{19}O_5$ 327.1227, found 327.1227.

*4.1.2.2. (Z)-2-(2,4-dibutoxybenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**5**).* The crude product was prepared according to procedure **A** starting from **3** (166 mg, 1.00 mmol) and 2,4-dibutoxybenzaldehyde [25] (376 mg, 1.5 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 98:2), the pure product (271 mg, 0.68 mmol, 68%) was obtained as a red powder. m.p. = 161–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.92 (bs, 2H), 8.05 (d, J = 8.8 Hz, 1H), 6.86 (s, 1H), 6.66 (dd, J = 8.8, 2.4 Hz, 1H), 6.62 (d, J = 2.4 Hz, 1H), 6.17 (d, J = 1.6 Hz, 1H), 6.05 (d, J = 1.6 Hz, 1H), 4.07 (m, 4H), 1.75 (m, 4H), 1.49 (m, 4H), 0.98 (t, J = 7.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.9 (C), 167.5 (C), 167.1 (C), 161.2 (C), 158.8 (C), 158.3 (C), 146.4 (C), 131.8 (CH), 113.5 (C), 106.8 (CH), 102.8 (C), 102.0 (CH), 99.3 (CH), 97.7 (CH), 90.4 (CH), 68.0 (CH_2), 67.4 (CH_2), 30.7 (2 × CH_2), 18.8 (CH_2), 18.7 (CH_2), 13.7 (2 × CH_3); LRMS (ESI+) *m/z* (%) 455 (16), 399 (100) $[M + H]^+$; HRMS (ESI+) *m/z* calc. for $C_{23}H_{27}O_6$ 399.1802, found 399.1802.

*4.1.2.3. (Z)-2-(4-dimethylaminobenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**6**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 4-dimethylaminobenzaldehyde (200 mg, 1.34 mmol). After purification by column chromatography on silica gel (EtOAc), the pure product (121 mg, 0.41 mmol, 68%) was obtained as a red powder. m.p. 252–253 °C; ^{1}H NMR (400 MHz,



Scheme 2. Synthesis of indolecarboxaldehyde derivatives. Reagents and conditions: (i) RX (*X* = Br or Cl), NaH 60% in oil, DMF, rt, 18–72 h, 41–99%.

DMSO-d_6) δ ppm 10.86 (bs, 2H), 7.74 (d, J = 8.0 Hz, 2H), 6.78 (d, J = 8.0 Hz, 2H), 6.54 (s, 1H), 6.18 (s, 1H), 6.05 (s, 1H), 3.00 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.8 (C), 167.2 (C), 166.8 (C), 158.0 (C), 150.7 (C), 145.3 (C), 132.4 (2 × CH), 119.5 (C), 112.0 (2 × CH), 110.0 (CH), 103.1 (C), 97.5 (CH), 90.3 (CH), 39.7 (2 × CH_3); LRMS (ESI+) *m/z* (%) 298 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{17}H_{16}NO_4$ 298.1074, found 298.1073.

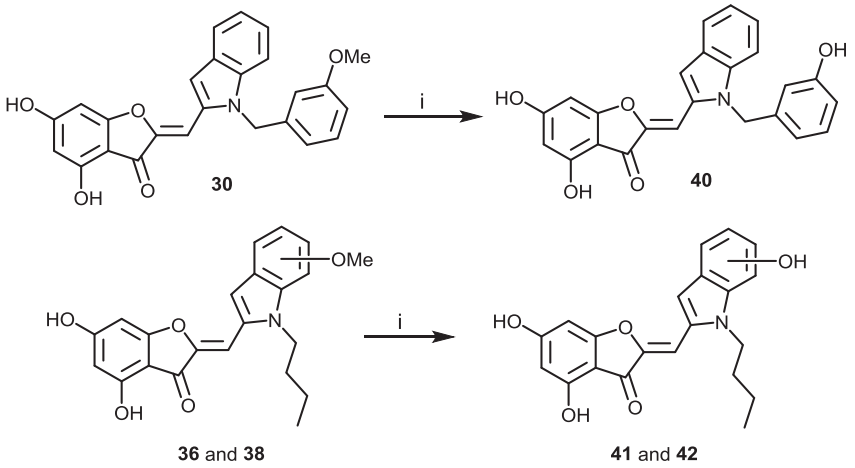
*4.1.2.4. (Z)-2-(4-piperidinylbenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**7**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 4-(1-piperidinyl) benzaldehyde (250 mg, 1.31 mmol). After purification by column chromatography on silica gel (EtOAc), the pure product (140 mg, 0.41 mmol, 69%) was obtained as a yellow powder. m.p. = 122–123 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.77 (bs, 2H), 7.72 (d, J = 8.0 Hz, 2H), 6.97 (d, J = 8.0 Hz, 2H), 6.52 (s, 1H), 6.19 (d, J = 1.6 Hz, 1H), 6.06 (d, J = 1.6 Hz, 1H), 3.32–3.21 (m, 4H), 1.66–1.59 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.8 (C), 167.3 (C), 166.7 (C), 158.0 (C), 151.4 (C), 145.6 (C), 132.3 (2 × CH), 121.1 (C), 114.5 (2 × CH), 109.6 (CH), 103.0 (C), 97.5 (CH), 90.3 (CH), 48.1 (2 × CH_2), 24.9 (2 × CH_2), 23.9 (CH_2); LRMS (ESI+) *m/z* (%) 338 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{20}H_{20}NO_4$ 338.1387, found 338.1387.

*4.1.2.5. (Z)-2-(4-Trifluoromethylbenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**8**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 4-trifluoromethylbenzaldehyde (200 mg, 1.15 mmol). After purification by column chromatography on silica gel (EtOAc), the pure product (138 mg, 0.43 mmol, 71%) was obtained as a yellow powder. m.p. 262–263 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.07 (bs, 2H), 8.08 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 2H), 6.69 (s, 1H), 6.23 (d, J = 1.2 Hz, 1H), 6.10 (d, J = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.8 (C), 167.9 (C), 167.8 (C), 158.7 (C), 149.0 (C), 136.5 (C), 131.0 (2 × CH), 128.5 (q, J = 32 Hz, CF_3), 125.6 (2 × CH), 122.7 (C), 106.0 (CH), 102.2 (C), 98.0 (CH), 90.7 (CH); LRMS (ESI+) *m/z* (%) 323 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{16}H_{10}O_4F_3$ 323.0526, found 323.0527.

*4.1.2.6. (Z)-2-[4-(2-thienyl)benzylidene]-4,6-dihydroxybenzofuran-3(2H)-one (**9**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 4-(2-thienyl) benzaldehyde (200 mg, 1.06 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 98:2), the pure product (134 mg, 0.40 mmol, 66%) was obtained as a red powder. m.p. 268–269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.01 (s, 2H), 7.94 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.63 (dd, J = 6.0, 4.0 Hz, 2H), 7.18 (dd, J = 6.0, 4.0 Hz, 1H), 6.63 (s, 1H), 6.23 (s, 1H), 6.08 (1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.9 (C), 167.7 (C), 167.5 (C), 158.5 (C), 147.8 (C), 142.7 (C), 134.2 (C), 131.5 (C), 131.4 (2 × CH), 128.7 (CH), 126.6 (CH), 125.7 (2 × CH), 124.6 (CH), 107.6 (CH), 102.5 (C), 97.8 (CH), 90.6 (CH); LRMS (ESI+) *m/z* (%) 337 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{19}H_{13}O_4S$ 337.0529, found 337.0528.

*4.1.2.7. (Z)-2-(4-Phenylbenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**10**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 4-phenylbenzaldehyde (200 mg, 1.10 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 98:2), the pure product (155 mg, 0.47 mmol, 78%) was obtained as a yellow powder. m.p. = 277–278 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.96 (s, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H), 7.76–7.71 (m, 2H), 7.53–7.46 (m, 2H), 7.43–7.36 (m, 1H), 6.67 (s, 1H), 6.25 (d, J = 1.6 Hz, 1H), 6.10 (d, J = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.9 (C), 167.8 (C), 167.4 (C), 158.4 (C), 147.8 (C), 140.5 (C), 139.3 (C), 131.5 (C), 131.2 (2 × CH), 129.0 (2 × CH), 127.9 (CH), 127.0 (2 × CH), 126.7 (2 × CH), 107.7 (CH), 102.5 (C), 97.8 (CH), 90.6 (CH); LRMS (ESI+) *m/z* (%) 331 (100) $[M + H]^+$; HRMS (ESI+) *m/z* calc. for $C_{21}H_{15}O_4$ 331.0965, found 331.0965.

*4.1.2.8. (Z)-2-(1-Naphthylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**11**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 1-naphthaldehyde (200 mg, 1.28 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 98:2), the pure product (148 mg, 0.49 mmol, 81%) was obtained as a yellow



Scheme 3. Hydrolysis of methoxy compounds. Reagents and conditions: (i) BBr_3, CH_2Cl_2, 0 °C to rt, 18–72 h, 93–96%.

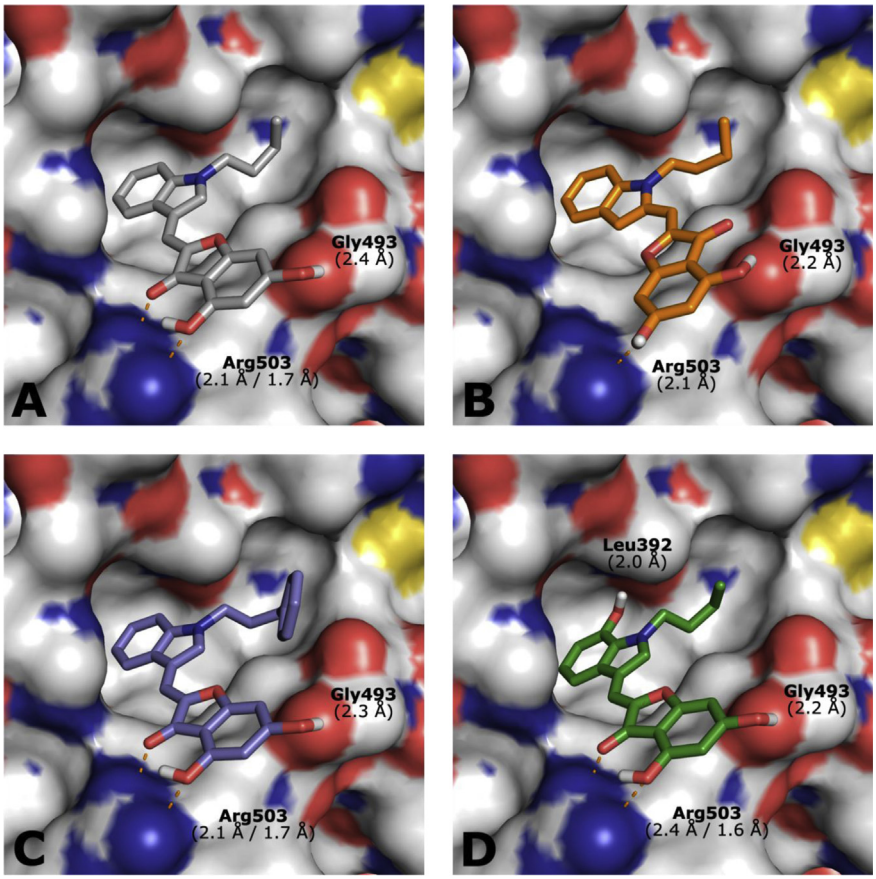


Fig. 2. Surface views of predicted binding modes in RdRp thumb pocket I of a reported X-ray structure (PDB code 2dxs) from docking studies with Autodock. (A) Compound **2**. (B) Compound **21**. (C) Compound **33**. (D) Compound **41**.

powder. m.p. >280 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.26 (bs, 2H), 8.27 (dd, J = 7.8, 7.8 Hz, 1H), 8.01 (dd, J = 7.8, 2.4 Hz, 2H), 7.68–7.58 (m, 3H), 7.26 (s, 2H), 6.31 (s, 1H), 6.28 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.8 (C), 167.8 (2 × C), 158.8 (C), 148.9 (C), 133.3 (C), 131.3 (C), 129.5 (CH), 128.9 (CH), 128.8 (CH), 128.2 (C), 127.1 (CH), 126.2 (CH), 125.8 (CH), 123.2 (CH), 103.1 (CH), 102.4 (C), 98.0 (CH), 90.6 (CH); LRMS (ESI+) *m/z* (%) 305 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{19}H_{13}O_4$ 305.0808, found 305.0808.

*4.1.2.9. (Z)-2-(2-naphthylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**12**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 2-naphthaldehyde (200 mg, 1.28 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 98:2), the pure product (161 mg, 0.53 mmol, 88%) was obtained as a yellow powder. m.p. = 276–277 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.02 (s, 2H), 8.41 (s, 1H), 8.08–7.93 (m, 4H), 7.57 (t, J = 6.4 Hz, 2H), 6.77 (s, 1H), 6.30 (s, 1H), 6.11 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 179.0 (C), 167.9 (C), 167.5 (C), 158.5 (C), 148.0 (C), 132.9 (C), 132.8 (C), 130.8 (CH), 130.1 (C), 128.4 (2 × CH), 127.6 (CH), 127.3 (CH), 127.2 (CH), 126.7 (CH), 108.2 (CH), 102.5 (C), 97.9 (CH), 90.7 (CH); LRMS (ESI+) *m/z* (%) 305 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{19}H_{13}O_4$ 305.0808, found 305.0808.

*4.1.2.10. (Z)-2-(2-quinolinemethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**13**).* The crude product was prepared according to procedure **A** starting from **3** (120 mg, 0.72 mmol) and 2-quinolinecarboxaldehyde (200 mg, 1.27 mmol). After purification by column chromatography on silica gel (EtOAc/MeOH 94:6), the pure product (186 mg, 0.59 mmol, 81%) was obtained as a yellow powder. m.p. >280 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.15 (s, 1H), 11.08 (s, 1H), 8.47 (d, J = 7.4 Hz, 1H), 8.25 (d, J = 7.4 Hz, 1H), 8.10–7.94 (m, 2H), 7.85–7.74 (m, 1H), 7.69–7.57 (m, 1H), 6.68 (s, 1H), 6.30 (s, 1H), 6.13 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.9 (C), 168.1 (C), 168.0 (C), 158.8 (C), 152.2 (C), 150.1 (C), 147.8 (C), 136.7 (CH), 130.1 (CH), 129.0 (CH), 127.8 (CH), 127.3 (CH), 126.8 (C), 122.4 (CH), 108.0 (CH), 102.1 (C), 98.0 (CH), 90.9 (CH); LRMS (ESI+) *m/z* (%) 306 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{18}H_{12}NO_4$ 306.0761, found 306.0761.

*4.1.2.11. (Z)-2-[(1H-benzo[d]imidazol-5-yl)methylene]-4,6-dihydroxybenzofuran-3(2H)-one (**14**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 1*H*-benzo[*d*]imidazole-5-carboxaldehyde (98 mg, 0.60 mmol), and was washed three times with distilled water to afford **14** as a yellow solid, which was analytically pure and used without further purification (133 mg, 0.45 mmol, 75%). m.p. >260 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.16 (bs, 2H), 9.47 (s, 1H), 8.37 (s, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 6.82 (s, 1H), 6.28 (s, 1H), 6.16 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.9 (C), 167.7 (2 × C), 158.6 (C), 148.0 (C), 141.9 (CH), 132.1 (C), 131.7 (C), 130.0 (C), 128.2 (CH), 116.3 (CH), 115.0 (CH), 107.5 (CH), 102.4 (C), 98.0 (CH), 90.6 (CH); LRMS (ESI−) *m/z* (%) 293 (100) $[M-H]^-$.

*4.1.2.12. (Z)-2-[(1H-imidazol-5-yl)methylene]-4,6-dihydroxybenzofuran-3(2H)-one (**15**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg,

0.60 mmol) and 1*H*-imidazole-5-carboxaldehyde (116 mg, 1.21 mmol), and was purified by column chromatography on silica gel (CH_2Cl_2/methanol 9:1) to afford **15** as a pure yellow solid (142 mg, 0.58 mmol, 97%). m.p. >260 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.87 (bs, 2H), 7.86 (s, 1H), 7.67 (s, 1H), 6.55 (s, 1H), 6.21 (s, 1H), 6.07 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.5 (C), 167.3 (C), 167.0 (C), 158.1 (C), 145.9 (C), 137.3 (CH), 130.1 (C), 125.0 (CH), 103.1 (C), 101.3 (CH), 97.6 (CH), 90.5 (CH). LRMS (ESI−) *m/z* (%) 243 (100) $[M-H]^-$.

*4.1.2.13. (Z)-2-[(1-methyl-1H-pyrrol-2-yl)methylene]-4,6-dihydroxybenzofuran-3(2H)-one (**16**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 1-methyl-1*H*-pyrrole-2-carboxaldehyde (132 mg, 1.21 mmol), and was purified by column chromatography on silica gel (CH_2Cl_2/methanol 19:1) to afford **16** as a pure brown solid (115 mg, 0.45 mmol, 75%). m.p. >240 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.78 (bs, 2H), 7.03 (m, 1H), 6.92 (m, 1H), 6.58 (s, 1H), 6.21 (m, 1H), 6.18 (d, J = 1.3 Hz, 1H), 6.05 (d, J = 1.3 Hz, 1H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.4 (C), 167.0 (C), 166.7 (C), 157.9 (C), 144.7 (C), 127.2 (C), 125.7 (CH), 115.6 (CH), 109.5 (CH), 103.3 (C), 98.3 (CH), 97.5 (CH), 90.4 (CH), 33.7 (CH_3); LRMS (ESI−) *m/z* (%) 256 (100) $[M-H]^-$.

*4.1.2.14. (Z)-2-[(Furan-2-yl)methylene]-4,6-dihydroxybenzofuran-3(2H)-one (**17**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and furan-2-carboxaldehyde (116 mg, 1.21 mmol), and was purified by column chromatography on silica gel (CH_2Cl_2/methanol 19:1) to afford **17** as a pure yellow solid (132 mg, 0.54 mmol, 90%). m.p. >230 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.97 (bs, 2H), 7.90 (d, J = 1.5 Hz, 1H), 7.04 (d, J = 3.4 Hz, 1H), 6.70 (dd, J = 3.4, 1.5 Hz, 1H), 6.54 (s, 1H), 6.19 (d, J = 1.7 Hz, 1H), 6.06 (d, J = 1.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.5 (C), 167.6 (C), 167.4 (C), 158.3 (C), 148.2 (C), 145.8 (C), 145.5 (CH), 115.7 (CH), 113.0 (CH), 102.8 (C), 97.8 (CH), 97.1 (CH), 90.6 (CH); LRMS (ESI−) *m/z* (%) 243 (100) $[M-H]^-$.

*4.1.2.15. (Z)-2-[(5-Hydroxymethyl)furan-2-yl)methylene]-4,6-dihydroxybenzofuran-3(2H)-one (**18**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 5-hydroxymethylfuran-2-carboxaldehyde (152 mg, 1.21 mmol), and was purified by column chromatography on silica gel (CH_2Cl_2/methanol 9:1) to afford **18** as a pure yellow solid (87 mg, 0.32 mmol, 53%). m.p. >240 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.97 (bs, 2H), 7.00 (d, J = 3.3 Hz, 1H), 6.52 (d, J = 3.3 Hz, 1H), 6.48 (s, 1H), 6.16 (d, J = 1.1 Hz, 1H), 6.05 (d, J = 1.1 Hz, 1H), 5.38 (bs, 1H), 4.46 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.6 (C), 167.4 (C), 158.0 (C), 147.5 (C), 145.7 (2 × C), 116.5 (CH), 110.3 (CH), 102.8 (C), 97.8 (CH), 97.1 (CH), 90.5 (CH), 55.8 (CH_2); LRMS (ESI−) *m/z* (%) 273 (100) $[M-H]^-$; Anal. calc. for $C_{14}H_{10}O_6 \cdot 0.25H_2O$: C, 60.32, H, 3.77, found: C, 59.68, H, 3.74.

*4.1.2.16. 2-(N-Butylindol-5-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**20**).* The crude product was prepared according to procedure **A** starting from **3** (166 mg, 1.00 mmol) and aldehyde **39a** (300 mg, 1.50 mmol). After purification by column chromatography on silica gel (Cyclohexane/EtOAc 2:1), the pure product (252 mg, 0.72 mmol, 72%) was obtained as a red powder. m.p. 209–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.81 (bs, 2H), 8.12 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H), 7.44 (d, J = 3.6 Hz, 1H), 6.71 (s, 1H), 6.53 (d, J = 2.5 Hz, 1H), 6.24 (s, 1H), 6.07 (s, 1H), 4.19 (t, J = 6.8 Hz, 2H), 1.81–1.67 (m, 2H), 1.30–1.12 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 179.0 (C), 167.6 (C), 167.0 (C), 158.2 (C), 146.1 (C), 136.1 (C), 130.0 (CH), 128.4 (C), 124.1 (CH), 124.0 (CH), 123.2 (CH), 110.7 (CH), 110.4 (C), 102.9 (C), 101.5 (CH), 97.6 (CH), 90.4 (CH), 45.3 (CH_2), 31.9 (CH_2), 19.4 (CH_2), 13.5 (CH_3); LRMS (ESI+) *m/z* (%) 193 (2), 350 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{21}H_{20}NO_4$ 350.1387, found 350.1390.

*4.1.2.17. 2-(N-Butylindol-2-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**21**).* The crude product was prepared according to procedure **A** starting from **3** (130 mg, 0.78 mmol) and aldehyde **39b** (235 mg, 1.17 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (148 mg, 0.42 mmol, 54%) was obtained as a red powder. m.p. 237–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.95 (bs, 2H), 7.64 (d, J = 6.8 Hz, 1H), 7.52 (d, J = 6.8 Hz, 1H), 7.30 (s, 1H), 7.26–7.15 (m, 1H), 7.13–6.99 (m, 1H), 6.74 (s, 1H), 6.28 (s, 1H), 6.10 (s, 1H), 4.52–4.23 (m, 2H), 1.78–1.50 (m, 2H), 1.37–1.07 (m, 2H), 1.03–0.70 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.4 (C), 167.3 (C), 158.4 (C), 148.0 (C), 137.5 (C), 131.0 (CH), 127.6 (C), 123.2 (CH), 121.0 (CH), 120.0 (CH), 110.2 (CH), 107.3 (C), 102.9 (CH), 97.8 (C), 97.1 (CH), 90.7 (CH), 42.2 (CH_2), 32.5 (CH_2), 19.6 (CH_2), 13.7 (CH_3); LRMS (ESI+) *m/z* (%) 350 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{21}H_{20}NO_4$ 350.1387, found 350.1389.

*4.1.2.18. 2-(N-Methylindol-2-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**22**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 1-methylindole-2-carboxaldehyde (300 mg, 2.04 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (121 mg, 0.40 mmol, 66%) was obtained as a red powder. m.p. 290–291 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.00 (bs, 1H), 10.95 (bs, 1H), 7.64 (d, J = 7.8 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.31 (s, 1H), 7.22 (dd, J = 8.2, 6.6 Hz,1H), 7.07 (dd, J = 7.8, 6.6 Hz,1H), 6.79 (s, 1H), 6.30 (s, 1H), 6.12 (s, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.5 (C), 167.4 (C), 158.4 (C), 148.0 (C), 138.0 (C), 131.8 (CH), 127.6 (C), 123.3 (CH), 121.0 (CH), 120.1 (CH), 110.2 (CH), 107.1 (C), 103.0 (CH), 97.9 (C), 97.3 (CH), 90.8 (CH), 29.6 (CH_3); LRMS (ESI+) *m/z* (%) 308 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{18}H_{14}NO_4$ 308.0917, found 308.0918.

*4.1.2.19. 2-(N-Methylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**23**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and 1-methylindole-3-carboxaldehyde (200 mg, 1.36 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (144 mg, 0.47 mmol, 78%) was obtained as a red powder. m.p. >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.68 (bs, 2H), 8.11 (s, 1H), 7.96 (d, J = 6.2 Hz, 1H), 7.53 (d, J = 6.5 Hz, 1H), 7.37–7.10 (m, 2H), 6.95 (s, 1H), 6.23 (s, 1H), 6.05 (s, 1H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.0 (C), 166.8 (C), 158.1 (C), 145.4 (C), 136.7 (C), 133.8 (CH), 127.1 (C), 122.5 (CH), 120.7 (CH), 118.9 (CH), 110.5 (CH), 107.3 (C), 103.7 (C), 102.1 (CH), 97.6 (CH), 90.3 (CH), 33.1 (CH_3); LRMS (ESI+) *m/z* (%) 308 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{18}H_{14}NO_4$ 308.0917, found 308.0920.

*4.1.2.20. 2-[N-(2-methylpropyl)indol-3-ylmethylene]-4,6-dihydroxybenzofuran-3(2H)-one (**24**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39c** (250 mg, 1.24 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (169 mg, 0.49 mmol, 81%) was obtained as orange crystals. m.p. 264–265 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.75 (s, 1H), 10.72 (s, 1H), 8.09 (s, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.24 (dd,

J = 8.1, 7.1 Hz, 1H), 7.19 (dd, J = 7.6, 7.1 Hz, 1H), 6.96 (s, 1H), 6.24 (s, 1H), 6.07 (s, 1H), 4.12 (d, J = 7.4 Hz, 2H), 2.25–2.13 (m, 1H), 0.88 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.0 (C), 166.5 (C), 157.9 (C), 145.3 (C), 136.3 (C), 133.2 (CH), 127.1 (C), 122.5 (CH), 120.6 (CH), 119.1 (CH), 110.9 (CH), 107.3 (C), 103.7 (C), 102.2 (CH), 97.5 (CH), 90.5 (CH), 53.2 (CH_2), 29.0 (CH), 19.8 (2 × CH_3); LRMS (ESI+) m/z (%) 350 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{21}H_{20}NO_4$ 350.1387, found 350.1388.

*4.1.2.21. 2-[N-(3-methylbut-2-enyl)indol-3-ylmethylene]-4,6-dihydroxybenzofuran-3(2H)-one (**25**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39d** (300 mg, 1.41 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (173 mg, 0.48 mmol, 80%) was obtained as a yellow powder. m.p. 251–252 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.75 (s, 1H), 10.73 (s, 1H), 8.11 (s, 1H), 7.97 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.25 (dd, J = 8.1, 6.9 Hz, 1H), 7.19 (dd, J = 7.7, 6.9 Hz, 1H), 6.96 (s, 1H), 6.23 (s, 1H), 6.06 (s, 1H), 5.43–5.30 (m, 1H), 4.91 (d, J = 6.7 Hz, 2H), 1.87 (s, 3H), 1.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.5 (C), 157.9 (C), 145.3 (C), 136.2 (C), 135.8 (CH), 132.5 (C), 127.3 (C), 122.4 (CH), 120.7 (CH), 119.8 (CH), 119.0 (CH), 110.7 (CH), 107.5 (C), 103.6 (C), 102.1 (CH), 97.5 (CH), 90.3 (CH), 44.2 (CH_2), 25.3 (CH_3), 17.9 (CH_3); LRMS (ESI+) m/z (%) 362 (100) $[M + H]^+$; HRMS (ESI+) m/z calc. for $C_{22}H_{20}NO_4$ 362.1387, found 362.1390.

*4.1.2.22. 2-[N-(3-Carboxypropyl)indol-3-ylmethylene]-4,6-dihydroxybenzofuran-3(2H)-one (**26**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39e** (191 mg, 0.90 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc/AcOH 2:1:0.005), the pure product (127 mg, 0.34 mmol, 56%) was obtained as a yellow powder. m.p. 253–254 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.21 (bs, 1H), 10.76 (bs, 2H), 8.12 (s, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.27 (dd, J = 8.1, 6.8 Hz, 1H), 7.19 (dd, J = 7.8, 6.8 Hz, 1H), 6.96 (s, 1H), 6.24 (s, 1H), 6.07 (s, 1H), 4.33 (t, J = 6.6 Hz, 2H), 2.26 (t, J = 7.2 Hz, 2H), 2.10–1.94 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 173.9 (C), 167.0 (C), 166.5 (C), 157.9 (C), 145.4 (C), 136.0 (C), 132.8 (CH), 127.2 (C), 122.6 (CH), 120.7 (CH), 119.2 (CH), 110.5 (CH), 107.6 (C), 103.6 (C), 102.1 (CH), 97.5 (CH), 90.4 (CH), 45.3 (CH_2), 30.7 (CH_2), 25.3 (CH_2); LRMS (ESI+) m/z (%) 380 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{21}H_{18}NO_6$ 380.1129, found 380.1131.

*4.1.2.23. 2-(N-cyclohexylmethylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**27**).* The crude product was prepared according to procedure **A** starting from **3** (207 mg, 1.25 mmol, 1.5 equiv.) and aldehyde **39f** (200 mg, 0.83 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (247 mg, 0.72 mmol, 87%) was obtained as orange crystals. m.p. 232–233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.75 (bs, 2H), 8.00 (s, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.25 (dd, J = 7.8, 7.2 Hz, 1H), 7.18 (dd, J = 7.5, 7.2 Hz, 1H), 6.94 (s, 1H), 6.23 (s, 1H), 6.05 (s, 1H), 4.14 (d, J = 7.2 Hz, 2H), 1.88–1.84 (m, 1H), 1.65–1.50 (m, 5H), 1.14–1.01 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.7 (C), 158.1 (C), 145.3 (C), 136.4 (C), 133.3 (CH), 127.0 (C), 122.4 (CH), 120.6 (CH), 119.0 (CH), 110.8 (CH), 107.3 (C), 103.7 (C), 102.0 (CH), 97.6 (CH), 90.3 (CH), 52.0 (CH_2), 38.2 (CH), 30.1 (2 × CH_2), 25.8 (CH_2), 25.1 (2 × CH_2); LRMS (ESI+) m/z (%) 390 (100) $[M + H]^+$; HRMS (ESI+) m/z calc. for $C_{24}H_{24}NO_4$ 390.1700, found 390.1699.

*4.1.2.24. 2-(N-Benzylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**28**).* The crude product was prepared according to procedure **A** starting from **3** (212 mg, 1.28 mmol) and aldehyde **39g** (200 mg, 0.85 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (186 mg, 0.48 mmol, 73%) was obtained as orange crystals. m.p. 280–281 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.72 (bs, 2H), 8.28 (s, 1H), 7.98 (d, J = 7.3 Hz, 1H), 7.51 (d, J = 7.7 Hz, 1H), 7.35–7.16 (m, 7H), 6.97 (s, 1H), 6.21 (s, 1H), 6.05 (s, 1H), 5.58 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.6 (C), 158.3 (C), 146.3 (CH), 145.5 (C), 137.4 (C), 135.9 (CH), 133.1 (C), 127.0 (C), 122.6 (2 × CH), 120.8 (2 × CH), 119.1 (CH), 118.4 (CH), 110.9 (CH), 109.2 (CH), 107.8 (C), 103.6 (C), 101.8 (CH), 97.5 (CH), 90.3 (CH), 49.5 (CH_2); LRMS (ESI+) m/z (%) 384 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{24}H_{18}NO_4$ 384.1230, found 384.1230.

*4.1.2.25. 2-(N-3-Methylbenzylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**29**).* The crude product was prepared according to procedure **A** starting from **3** (200 mg, 1.20 mmol) and aldehyde **39h** (200 mg, 0.80 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (171 mg, 0.43 mmol, 54%) was obtained as orange crystals. m.p. 262–263 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.74 (bs, 2H), 8.28 (s, 1H), 8.00 (d, J = 7.1 Hz, 1H), 7.52 (d, J = 7.4 Hz, 1H), 7.23–7.04 (m, 6H), 6.99 (s, 1H), 6.23 (s, 1H), 6.08 (s, 1H), 5.54 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.7 (C), 158.3 (C), 145.5 (C), 137.8 (CH), 137.3 (C), 136.0 (C), 133.2 (CH), 128.6 (CH), 128.2 (C), 127.6 (CH), 127.4 (C), 124.2 (CH), 122.6 (CH), 120.8 (CH), 119.1 (CH), 110.9 (CH), 107.8 (C), 103.7 (C), 101.9 (CH), 97.6 (CH), 90.3 (CH), 49.6 (CH_2), 21.0 (CH_3); LRMS (ESI+) m/z (%) 416 (18) $[M+H_2O+H]^+$, 398 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{25}H_{20}NO_4$ 398.1387, found 398.1386.

*4.1.2.26. 2-(N-3-Methoxybenzylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**30**).* The crude product was prepared according to procedure **A** starting from **3** (150 mg, 0.90 mmol) and aldehyde **39i** (450 mg, 1.78 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (268 mg, 0.65 mmol, 72%) was obtained as a yellow powder. m.p. 248–249 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.77 (s, 1H), 10.74 (s, 1H), 8.28 (s, 1H), 7.99 (d, J = 7.4 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.30–7.12 (m, 3H), 6.97 (s, 1H), 6.91–6.80 (m, 2H), 6.77 (d, J = 7.7 Hz, 1H), 6.22 (s, 1H), 6.07 (s, 1H), 5.54 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.0 (C), 166.6 (C), 159.4 (C), 157.9 (C), 145.6 (C), 139.1 (CH), 136.0 (C), 133.3 (C), 129.9 (CH), 127.4 (C), 122.7 (CH), 120.9 (CH), 119.1 (2 × CH), 113.2 (CH), 112.5 (CH), 111.0 (CH), 107.9 (C), 103.6 (C), 102.0 (CH), 97.5 (CH), 90.4 (CH), 55.1 (CH_3), 49.5 (CH_2); LRMS (ESI+) m/z (%) 414 (100) $[M+H]^+$, 338 (2); HRMS (ESI+) m/z calc. for $C_{25}H_{20}NO_5$ 414.1356, found 414.1338.

*4.1.2.27. 2-(N-3-Bromobenzylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**31**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and *N*-(3-bromobenzyl)indole-3-carboxaldehyde [30] (400 mg, 1.27 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (171 mg, 0.37 mmol, 62%) was obtained as a yellow powder. m.p. 151–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.78 (s, 1H), 10.75 (s, 1H), 8.32 (s, 1H), 7.99 (d, J = 7.3 Hz, 1H), 7.60–7.40 (m, 3H), 7.35–7.13 (m, 4H), 6.98 (s, 1H), 6.22 (d, J = 1.5 Hz, 1H), 6.08 (d, J = 1.5 Hz, 1H), 5.60 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.4 (C), 167.0 (C), 166.6 (C), 158.0 (C), 145.8 (C), 136.3 (CH), 136.1

(C), 133.3 (C), 132.9 (CH), 129.7 (CH), 128.3 (C), 128.2 (CH), 127.3 (CH), 122.9 (C), 122.0 (CH), 121.1 (CH), 119.3 (CH), 110.7 (CH), 108.3 (C), 103.6 (C), 101.8 (CH), 97.6 (CH), 90.4 (CH), 49.8 (CH_2); LRMS (ESI+) *m/z* (%) 464 (100) [$M(^{81}Br)$+H]$^+$, 462 (100) [$M(^{79}Br)$+H]$^+$, 314 (3), 193 (2); HRMS (ESI+) *m/z* calc. for $C_{24}H_{17}BrNO_4$ 462.0335, found 462.0334.

*4.1.2.28. 2-(N-2-phenylethylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**32**).* The crude product was prepared according to procedure **A** starting from **3** (200 mg, 1.20 mmol) and aldehyde **39j** (200 mg, 0.80 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (145 mg, 0.36 mmol, 46%) was obtained as orange crystals. m.p. >240 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.61 (bs, 2H), 8.02 (s, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.61 (d, J = 8.1 Hz, 1H), 7.30 (m, 4H), 7.26–7.20 (m, 3H), 6.92 (s, 1H), 6.23 (s, 1H), 6.08 (s, 1H), 4.57 (t, J = 7.3 Hz, 2H), 3.15 (t, J = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (2 × C), 158.3 (C), 145.4 (C), 138.4 (CH), 135.8 (C), 132.8 (CH), 128.9 (2 × CH), 128.3 (2 × CH), 127.1 (C), 126.5 (C), 122.4 (C), 120.6 (CH), 119.0 (CH), 118.0 (CH), 110.6 (CH), 107.4 (C), 101.9 (CH), 97.6 (CH), 90.4 (CH), 47.4 (CH_2), 35.8 (CH_2); LRMS (ESI+) *m/z* (%) 416 (33) [M + H_2O + H]$^+$, 398 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{25}H_{20}NO_4$ 398.1387, found 398.1385.

*4.1.2.29. 2-(N-3-phenylpropylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**33**).* The crude product was prepared according to procedure **A** starting from **3** (284 mg, 1.71 mmol) and aldehyde **39k** (300 mg, 1.14 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) and recrystallization in acetonitrile, the pure product (304 mg, 0.74 mmol, 65%) was obtained as orange crystals. m.p. 211–212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.76 (bs, 2H), 8.00 (s, 1H), 7.85 (d, J = 7.3 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.17–7.08 (m, 7H), 6.84 (s, 1H), 6.13 (s, 1H), 5.96 (s, 1H), 4.21 (s, 2H), 2.50 (s, 2H), 2.02 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.6 (C), 158.1 (C), 145.3 (C), 141.0 (CH), 135.9 (C), 132.8 (CH), 128.4 (2 × CH), 128.2 (2 × CH), 127.2 (C), 125.9 (C), 122.5 (C), 120.7 (CH), 119.1 (CH), 119.0 (CH), 110.5 (CH), 107.5 (C), 102.0 (CH), 97.5 (CH), 90.3 (CH), 45.7 (CH_2), 32.2 (CH_2), 31.2 (CH_2); LRMS (ESI+) *m/z* (%) 412 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{26}H_{22}NO_4$ 412.1543, found 412.1543.

*4.1.2.30. 2-(N-Butyl-2-methylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**34**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39l** (300 mg, 1.39 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (122 mg, 0.34 mmol, 56%) was obtained as a red powder. m.p. 158–159 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.71 (bs, 2H), 8.23 (d, J = 6.8 Hz, 1H), 7.50 (d, J = 6.8 Hz, 1H), 7.30–7.07 (m, 2H), 6.80 (s, 1H), 6.20 (s, 1H), 6.07 (s, 1H), 4.34–4.10 (m, 2H), 2.56 (s, 3H), 1.76–1.55 (m, 2H), 1.43–1.22 (m, 2H), 0.90 (t, J = 6.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.4 (C), 166.6 (C), 166.4 (C), 157.8 (C) 143.5 (C), 141.9 (C), 136.6 (C), 125.4 (C), 121.8 (CH), 121.1 (CH), 120.5 (CH), 110.0 (CH), 105.7 (C), 103.5 (CH), 103.4 (C), 97.4 (CH), 90.1 (CH), 42.8 (CH_2), 31.5 (CH_2), 19.5 (CH_2), 13.6 (CH_3), 10.6 (CH_3); LRMS (ESI+) *m/z* (%) 364 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{22}H_{22}NO_4$ 364.1543, found 364.1546.

*4.1.2.31. 2-(N-Butyl-7-methylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**35**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39m** (300 mg, 1.39 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (133 mg, 0.36 mmol, 61%) was obtained as a red powder. m.p. 232–233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.76 (s, 2H), 8.07 (s, 1H), 7.76 (d, J = 7.3 Hz, 1H), 7.19–6.79 (m, 3H), 6.27 (s, 1H), 6.09 (s, 1H), 4.56–4.20 (m, 2H), 2.68 (s, 3H), 1.85–1.58 (m, 2H), 1.42–1.20 (m, 2H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.0 (C), 166.5 (C), 157.9 (C), 145.5 (C), 134.3 (CH), 134.2 (C), 128.5 (CH), 125.6 (C), 121.3 (CH), 120.8 (C), 116.9 (CH), 107.2 (C), 103.7 (C), 101.8 (CH), 97.5 (CH), 90.5 (CH), 48.4 (CH_2), 34.3 (CH_2), 19.4 (CH_2), 15.2 (CH_3), 13.6 (CH_3); LRMS (ESI+) *m/z* (%) 364 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{22}H_{22}NO_4$ 364.1543, found 364.1547.

*4.1.2.32. 2-(N-butyl-7-methoxyindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**36**).* The crude product was prepared according to procedure **A** starting from **3** (38 mg, 0.23 mmol) and aldehyde **39n** (70 mg, 0.30 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (68 mg, 0.18 mmol, 78%) was obtained as a red powder. m.p. 247–248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.74 (s, 1H), 10.72 (s, 1H), 8.00 (s, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.08 (dd, J = 7.9, 7.9 Hz, 1H), 6.88 (s, 1H), 6.79 (d, J = 7.9 Hz, 1H), 6.24 (d, J = 1.7 Hz, 1H), 6.06 (d, J = 1.7 Hz, 1H), 4.45 (t, J = 7.0 Hz, 2H), 3.91 (s, 3H), 1.82–1.69 (m, 2H), 1.35–1.21 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.9 (C), 166.5 (C), 157.9 (C) 147.3 (C), 145.4 (C), 133.6 (CH), 129.7 (CH), 125.1 (C), 121.5 (CH), 111.4 (CH), 107.3 (C), 104.0 (C), 103.6 (C), 102.0 (CH), 97.5 (CH), 90.5 (CH), 55.6 (CH_3), 48.6 (CH_2), 33.7 (CH_2), 19.3 (CH_2), 13.6 (CH_3); LRMS (ESI+) *m/z* (%) 380 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{22}H_{22}NO_5$ 380.1492, found 380.1496.

*4.1.2.33. 2-(N-butyl-5-methylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**37**).* The crude product was prepared according to procedure **A** starting from **3** (100 mg, 0.60 mmol) and aldehyde **39o** (350 mg, 1.63 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (177 mg, 0.49 mmol, 81%) was obtained as a yellow powder. m.p. 157–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.75 (bs, 2H), 8.06 (s, 1H), 7.72 (s, 1H), 7.39 (d, J = 6.0 Hz, 1H), 7.05 (d, J = 5.7 Hz, 1H), 6.96 (s, 1H), 6.28 (s, 1H), 6.10 (s, 1H), 4.33–4.02 (m, 2H), 2.41 (s, 3H), 1.81–1.57 (m, 2H), 1.40–1.06 (m, 2H), 0.99–0.64 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.4 (C), 166.9 (C), 166.6 (C), 158.0 (C), 145.2 (C), 134.4 (CH), 132.7 (C), 129.6 (CH), 127.5 (CH), 124.0 (C), 118.6 (CH), 110.3 (C), 106.9 (CH), 103.8 (C), 102.3 (C), 97.6 (CH), 90.4 (CH), 45.9 (CH_2), 31.9 (CH_2), 21.2 (CH_2), 19.5 (CH_3), 13.5 (CH_3); LRMS (ESI+) *m/z* (%) 364 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{22}H_{22}NO_4$ 364.1543, found 364.1547.

*4.1.2.34. 2-(N-butyl-5-methoxyindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**38**).* The crude product was prepared according to procedure **A** starting from **3** (280 mg, 1.69 mmol) and aldehyde **39p** (1.10 g, 4.76 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 1:1), the pure product (544 mg, 1.43 mmol, 85%) was obtained as a yellow powder. m.p. 250–251 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.73 (s, 1H), 10.71 (s, 1H), 8.06 (s, 1H), 7.54 (s, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.01 (s, 1H), 6.86 (d, J = 7.5 Hz, 1H), 6.23 (s, 1H), 6.07 (s, 1H), 4.24 (t, J = 6.4 Hz, 2H), 3.84 (s, 3H), 1.84–1.64 (m, 2H), 1.37–1.13 (m, 2H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 166.8 (C), 166.4 (C), 157.8 (C), 154.8 (C), 144.9 (C), 133.2 (CH), 131.0 (CH), 127.9 (CH), 112.7 (C), 111.5 (CH), 107.3 (C), 103.8 (C), 102.9 (C), 100.9 (CH), 97.4 (CH), 90.4 (CH), 55.9 (CH_3), 46.0 (CH_2), 31.9 (CH_2), 19.5 (CH_2), 13.6 (CH_3); LRMS (ESI+) *m/z* (%) 380 (100) [M+H]$^+$; HRMS (ESI+) *m/z* calc. for $C_{22}H_{22}NO_5$ 380.1492, found 380.1496.

*4.1.3. General procedure B for the synthesis of N-alkyl-indole-3-carboxaldehyde (**39a**–**39p**)*

To a suspension of NaH 60% in oil (2.25 equiv.) in dry DMF (0.8 mL/mmol) was added, at 0 °C and under nitrogen atmosphere, a solution of indolecarboxaldehyde (1 equiv.) in dry DMF (2.5 mL/mmol). After stirring for 30 min at rt, alkyl halide (1.0–3.0 equiv.) was slowly added. After stirring overnight, the reaction was quenched by addition of water and the product was extracted with diethyl ether. The organic layer was dried over $MgSO_4$, filtered off and concentrated under vacuum. The crude product was purified by column chromatography on silica gel.

*4.1.3.1. N-butylindole-5-carboxaldehyde (**39a**).* The crude product was prepared according to procedure **B** starting from indole-5-carboxaldehyde (200 mg, 1.37 mmol) and 1-bromobutane (377 mg, 2.75 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (134 mg, 0.67 mmol, 49%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.00 (s, 1H), 8.13 (d, J = 0.9 Hz, 1H), 7.76 (dd, J = 8.6, 1.6 Hz, 1H), 7.39 (d, J = 8.6 Hz, 1H), 7.17 (d, J = 3.2 Hz, 1H), 6.63 (dd, J = 3.2, 0.9 Hz, 1H), 4.14 (t, J = 7.1 Hz, 2H), 1.86–1.76 (m, 2H), 1.39–1.26 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 192.7 (CH), 139.5 (C), 129.9 (CH), 129.5 (C), 128.5 (C), 126.8 (CH), 121.8 (CH), 110.1 (CH), 103.4 (CH), 46.7 (CH_2), 32.5 (CH_2), 20.3 (CH_2), 13.9 (CH_3).

*4.1.3.2. N-butylindole-2-carboxaldehyde (**39b**).* The crude product was prepared according to procedure **B** starting from indole-2-carboxaldehyde (200 mg, 1.37 mmol) and 1-bromobutane (377 mg, 2.70 mmol). After purification by column chromatography on silica gel (cyclohexane), the pure product (116 mg, 0.58 mmol, 42%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.86 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.43–7.35 (m, 2H), 7.24 (s, 1H), 7.18–7.12 (m, 1H), 4.54 (t, J = 7.4 Hz, 2H), 1.81–1.69 (m, 2H), 1.40–1.29 (m, 2H), 0.92 (d, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 182.8 (CH), 140.5 (C), 135.6 (C), 127.0 (CH), 126.6 (C), 123.6 (CH), 121.0 (CH), 118.1 (CH), 110.9 (CH), 44.8 (CH_2), 32.8 (CH_2), 20.3 (CH_2),14.0 (CH_3).

*4.1.3.3. N-(2-methylpropyl)-indole-3-carboxaldehyde (**39c**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (603 mg, 4.15 mmol) and 1-bromo-2-methylpropane (1.27 g, 9.35 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (600 mg, 2.98 mmol, 72%) was obtained as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.97 (s, 1H), 8.39–8.33 (m, 1H), 7.62 (s, 1H), 7.37–7.27 (m, 3H), 3.87 (d, J = 7.4 Hz, 2H), 2.22–2.13 (m, 1H), 0.89 (d, J = 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.3 (CH), 139.1 (CH), 137.3 (C), 125.1 (C), 123.7 (CH), 122.5 (CH), 121.8 (CH), 117.7 (C), 110.3 (CH), 54.4 (CH_2), 28.9 (CH), 19.9 (2 × CH_3).

*4.1.3.4. N-(3-Methylbut-2-enyl)-indole-3-carboxaldehyde (**39d**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (726 mg, 5.00 mmol) and 1-bromo-3-methylbut-2-ene (1.49 g, 10.00 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (885 mg, 4.15 mmol, 83%) was obtained as a gray powder. m.p. 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.94 (s, 1H), 8.32–8.24 (m, 1H), 7.69 (s, 1H), 7.37–7.25 (m, 3H), 5.43–5.33 (m, 1H), 4.68 (d, J = 7.0 Hz, 2H), 1.81 (s, 3H), 1.80 (d, J = 0.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.6 (CH), 139.1 (C), 138.0 (CH), 137.4 (C), 125.7 (C), 124.0 (CH), 123.0 (CH), 122.1 (CH), 118.1 (C), 117.9 (CH), 110.3 (CH), 44.9 (CH_2), 25.8 (CH_3), 18.3 (CH_3).

*4.1.3.5. N-(3-Cyanopropyl)-indole-3-carboxaldehyde (**39e**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (650 mg, 4.48 mmol) and 5-chloropentanenitrile (1.00 g, 9.66 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (809 mg, 3.81 mmol, 85%) was obtained as a light gray powder. m.p. 77–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.98 (s, 1H), 8.35–8.24 (m, 1H), 7.72 (s, 1H), 7.41–7.26 (m, 3H), 4.36 (t, J = 6.6 Hz, 2H), 2.38–2.13 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.7 (CH), 138.1 (CH), 137.0 (C), 125.7 (C), 124.6 (CH), 123.5 (CH), 122.6 (CH), 118.9 (C), 118.4 (C), 109.9 (CH), 45.4 (CH_2), 25.7 (CH_2), 14.8 (CH_2).

*4.1.3.6. N-(Cyclohexylmethyl)-indole-3-carboxaldehyde (**39f**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (300 mg, 2.07 mmol) and bromomethylcyclohexane (549 mg, 3.10 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (260 mg, 1.08 mmol, 52%) was obtained as a pink powder. m.p. 94–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.00 (s, 1H), 8.34–8.29 (m, 1H), 7.66 (s, 1H), 7.40–7.28 (m, 3H), 3.98 (d, J = 7.2 Hz, 2H), 1.95–1.82 (m, 1H), 1.77–1.60 (m, 5H), 1.29–1.13 (m, 3H), 1.08–0.96 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.6 (CH), 139.1 (CH), 137.7 (C), 125.5 (C), 124.0 (CH), 122.9 (CH), 122.2 (CH), 118.0 (C), 110.5 (CH), 53.9 (CH_2), 38.4 (CH), 31.1 (2 × CH_2), 26.3 (2 × CH_2), 25.7 (CH_2).

*4.1.3.7. N-benzyl-indole-3-carboxaldehyde (**39g**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (610 mg, 4.20 mmol) and benzyl bromide (1.62 g, 9.46 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (0.98 g, 4.17 mmol, 99%) was obtained as a white solid. m.p. 111–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.02 (s, 1H), 8.42–8.38 (m, 1H), 7.73 (s, 1H), 7.43–7.30 (m, 6H), 7.25–7.20 (m, 2H), 5.37 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.7 (CH), 138.8 (CH), 137.6 (C), 135.5 (C), 129.2 (2 × CH), 128.5 (CH), 127.3 (2 × CH), 125.6 (C), 124.3 (CH), 123.2 (CH), 122.2 (CH), 118.6 (C), 110.6 (CH), 51.0 (CH_2).

*4.1.3.8. N-(3-Methylbenzyl)-indole-3-carboxaldehyde (**39h**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (610 mg, 4.20 mmol) and 3-methylbenzylbromide (1.78 g, 9.46 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (900 mg, 3.61 mmol, 86%) was obtained as a yellow solid. m.p. 78–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.00 (s, 1H), 8.47–8.42 (m, 1H), 7.69 (s, 1H), 7.40–7.25 (m, 4H), 7.19 (d, J = 7.6 Hz, 1H), 7.07 (s, 1H), 7.02 (d, J = 7.6 Hz, 1H), 5.27 (s, 2H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.5 (CH), 138.8 (CH), 138.7 (C), 137.4 (C), 135.3 (C), 129.0 (CH), 128.9 (CH), 127.9 (CH), 125.4 (C), 124.3 (CH), 124.0 (CH), 122.9 (CH), 122.0 (CH), 118.2 (C), 110.5 (CH), 50.7 (CH_2), 21.3 (CH_3).

*4.1.3.9. N-(3-Methoxybenzyl)-indole-3-carboxaldehyde (**39i**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (726 mg, 5.00 mmol) and 3-methoxybenzylbromide (1.01 g, 5.00 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (1.06 g, 4.00 mmol, 80%) was obtained as a yellow powder. m.p. 110–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.98 (s, 1H), 8.36–8.29 (m, 1H), 7.69 (s, 1H), 7.35–7.25 (m, 4H), 6.85 (dd, J = 8.2, 2.3 Hz, 1H), 6.77–6.73 (m, 1H), 6.72–6.69 (m, 1H), 5.29 (s, 2H), 3.75 (s, 3H); ^{13}C NsMR (100 MHz, $CDCl_3$) δ ppm 184.7 (CH), 160.3 (C), 138.7 (CH), 137.6 (C), 137.0 (C), 130.3 (CH), 125.6 (C), 124.3

(CH), 123.2 (CH), 122.3 (CH), 119.6 (CH), 118.6 (C), 113.5 (CH), 113.4 (CH), 110.5 (CH), 55.4 (CH_3), 51.0 (CH_2).

*4.1.3.10. N-(2-Phenylethyl)-indole-3-carboxaldehyde (**39j**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (603 mg, 4.15 mmol) and 2-(bromoethyl) benzene (1.74 g, 9.35 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (420 mg, 1.68 mmol, 41%) was obtained as an orange solid. m.p. 72–73 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.90 (s, 1H), 8.45–8.41 (m, 1H), 7.42–7.37 (m, 4H), 7.33–7.26 (m, 3H), 7.10–7.04 (m, 2H), 4.37 (t, J = 7.1 Hz, 2H), 3.15 (t, J = 7.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.4 (CH), 138.9 (CH), 137.4 (C), 136.8 (CH), 128.7 (2 × CH), 128.6 (2 × CH), 126.9 (CH), 125.3 (C), 123.9 (CH), 122.8 (CH), 122.1 (CH), 117.8 (C), 110.0 (C), 48.6 (CH_2), 35.9 (CH_2).

*4.1.3.11. N-(3-Phenylpropyl)-indole-3-carboxaldehyde (**39k**).* The crude product was prepared according to procedure **B** starting from indole-3-carboxaldehyde (603 mg, 4.15 mmol) and 3-(bromopropyl)benzene (1.88 g, 9.35 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (902 mg, 3.43 mmol, 83%) was obtained as an orange solid. m.p. 49–50 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.03 (s, 1H), 8.49–8.46 (m, 1H), 7.62 (s, 1H), 7.39–7.26 (m, 6H), 7.21–7.17 (m, 2H), 4.08 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 7.4 Hz, 2H), 2.21–2.11 (dt, J = 7.4, 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.2 (CH), 140.1 (CH), 138.5 (C), 136.9 (C), 128.4 (2 × CH), 128.1 (2 × CH), 126.1 (CH), 125.1 (C), 123.7 (CH), 122.6 (CH), 121.8 (CH), 117.8 (C), 110.0 (CH), 46.0 (CH_2), 32.4 (CH_2), 30.6 (CH_2).

*4.1.3.12. N-Butyl-2-methylindole-3-carboxaldehyde (**39l**).* The crude product was prepared according to procedure **B** starting from 2-methylindole-3-carboxaldehyde (418 mg, 2.63 mmol) and 1-bromobutane (330 mg, 2.41 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 8:2), the pure product (308 mg, 1.43 mmol, 59%) was obtained as a yellow powder. m.p. 56–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 10.08 (s, 1H), 8.27–8.21 (m, 1H), 7.33–7.24 (m, 3H), 4.10 (t, J = 7.5 Hz, 2H), 2.69 (s, 3H), 1.82–1.70 (m, 2H), 1.46–1.34 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 183.9 (CH), 148.4 (C), 136.7 (C), 126.1 (C), 123.5 (CH), 123.3 (CH), 121.3 (CH), 114.3 (C), 109.9 (CH), 43.6 (CH_2), 31.9 (CH_2), 20.5 (CH_2), 14.0 (CH_3), 10.9 (CH_3).

*4.1.3.13. N-Butyl-7-methylindole-3-carboxaldehyde (**39m**).* The crude product was prepared according to procedure **B** starting from 7-methylindole-3-carboxaldehyde (400 mg, 2.51 mmol) and 1-bromobutane (685 mg, 5.00 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (372 mg, 1.73 mmol, 69%) was obtained as a yellow powder. m.p. 90–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.94 (s, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.63 (s, 1H), 7.17 (dd, J = 7.5, 7.5 Hz, 1H), 7.06–7.01 (m, 1H), 4.34 (t, J = 7.4 Hz, 2H), 2.70 (s, 3H), 1.89–0.1.76 (m, 2H), 1.46–1.31 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.5 (CH), 140.1 (CH), 136.1 (C), 127.3 (CH), 126.8 (C), 123.2 (CH), 121.5 (C), 120.3 (CH), 117.9 (C), 49.8 (CH_2), 34.3 (CH_2), 20.1 (CH_2), 19.8 (CH_3), 13.8 (CH_3).

*4.1.3.14. N-Butyl-7-methoxyindole-3-carboxaldehyde (**39n**).* The crude product was prepared according to procedure **B** starting from 7-methoxyindole-3-carboxaldehyde (45 mg, 0.25 mmol) and 1-bromobutane (70 mg, 0.51 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 95:5), the pure product (54 mg, 0.23 mmol, 91%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.94 (s, 1H), 7.87 (dd, J = 7.9, 0.8 Hz, 1H), 7.56 (s, 1H), 7.17 (dd, J = 7.9, 7.9 Hz, 1H), 6.72 (dd, J = 7.9, 0.8 Hz, 1H), 4.38 (t, J = 7.2 Hz, 2H), 3.92 (s, 3H), 1.86–1.76 (m, 2H), 1.39–1.27 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.6 (CH), 147.6 (CH), 139.3 (C), 128.1 (C), 126.9 (C), 123.7 (CH), 118.0 (C), 114.6 (CH), 105.0 (CH), 55.6 (CH_3), 50.5 (CH_2), 33.9 (CH_2), 19.9 (CH_2), 13.8 (CH_3).

*4.1.3.15. N-Butyl-5-methylindole-3-carboxaldehyde (**39o**).* The crude product was prepared according to procedure **B** starting from 5-methylindole-3-carboxaldehyde (400 mg, 2.51 mmol) and 1-bromobutane (685 mg, 5.00 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (405 mg, 1.88 mmol, 75%) was obtained as an orange powder. m.p. 55–56 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.93 (s, 1H), 8.10 (s, 1H), 7.64 (s, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.14 (dd, J = 8.4, 1.5 Hz, 1H), 4.12 (t, J = 7.1 Hz, 2H), 2.46 (s, 3H), 1.90–1.78 (m, 2H), 1.41–1.28 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.6 (CH), 138.6 (C), 135.8 (C), 132.8 (CH), 125.9 (C), 125.6 (CH), 122.1 (CH), 117.8 (C), 109.9 (CH), 47.2 (CH_2), 31.9 (CH_2), 21.6 (CH_3), 20.2 (CH_2), 13.7 (CH_3).

*4.1.3.16. N-Butyl-5-methoxyindole-3-carboxaldehyde (**39p**).* The crude product was prepared according to procedure **B** starting from 5-methoxyindole-3-carboxaldehyde (200 mg, 1.14 mmol) and 1-bromobutane (312 mg, 2.28 mmol). After purification by column chromatography on silica gel (cyclohexane/EtOAc 9:1), the pure product (243 mg, 1.05 mmol, 92%) was obtained as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.91 (s, 1H), 7.77 (d, J = 2.5 Hz, 1H), 7.64 (s, 1H), 7.23 (d, J = 8.9 Hz, 1H), 6.94 (dd, J = 8.9, 2.5 Hz, 1H), 4.12 (t, J = 7.1 Hz, 2H), 3.88 (s, 3H), 1.90–1.79 (m, 2H), 1.40–1.29 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 184.6 (CH), 156.9 (C), 138.6 (CH), 132.3 (C), 126.4 (C), 117.9 (C), 114.7 (CH), 111.1 (CH), 103.6 (CH), 56.0 (CH_3), 47.4 (CH_2), 32.0 (CH_2), 20.2 (CH_2), 13.8 (CH_3).

*4.1.4. General procedure C for the synthesis of compounds (**40**–**42**)*

To a solution of derivatives **30**, **36** or **38** in anhydrous CH_2Cl_2 (10 mL/mmol) was added BBr_3 (20 equiv.) at 0 °C. The solution was stirred at rt for 18–72 h, then EtOAc and iced water were added. After stirring for 0.5 h, the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered off and concentrated under reduced pressure to afford the expected crude product.

*4.1.4.1. 2-(N-3-hydroxybenzylindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**40**).* The crude product was prepared according to procedure **C** starting from compound **30** (120 mg, 0.29 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 95:5), the pure product (108 mg, 0.27 mmol, 93%) was obtained as a red powder. m.p. >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.77 (s, 1H), 10.76 (s, 1H), 9.41 (s, 1H), 8.28 (s, 1H), 7.99 (d, J = 6.9 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.31–7.05 (m, 3H), 6.99 (s, 1H), 6.71 (d, J = 7.2 Hz, 1H), 6.64 (d, J = 7.2 Hz, 1H), 6.55 (s, 1H), 6.23 (s, 1H), 6.07 (s, 1H), 5.50 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.3 (C), 167.0 (C), 166.6 (C), 157.9 (C), 157.6 (C), 145.5 (C), 138.9 (CH), 136.0 (C), 133.3 (C), 129.7 (CH), 127.4 (C), 122.7 (CH), 120.8 (CH), 119.1 (CH), 117.6 (CH), 114.5 (CH), 113.5 (CH), 111.0 (CH), 107.8 (C), 103.6 (C), 102.0 (CH), 97.5 (CH), 90.4 (CH), 49.5 (CH_2); LRMS (ESI+) *m/z* (%) 400 (100) $[M+H]^+$; HRMS (ESI+) *m/z* calc. for $C_{24}H_{18}NO_5$ 400.1179, found 400.1179.

*4.1.4.2. 2-(N-Butyl-7-hydroxyindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**41**).* The crude product was prepared according to procedure **C** starting from compound **36** (50 mg, 0.13 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1), the pure product (46 mg, 0.13 mmol, 95%)

was obtained as a brownish red powder. m.p. 149–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.72 (s, 1H), 10.71 (s, 1H), 9.88 (s, 1H), 7.97 (s, 1H), 7.32 (d, J = 7.9 Hz, 1H), 6.94 (dd, J = 7.9, 7.6 Hz, 1H), 6.86 (s, 1H), 6.61 (d, J = 7.6 Hz, 1H), 6.23 (d, J = 1.2 Hz, 1H), 6.05 (d, J = 1.2 Hz, 1H), 4.48 (t, J = 6.9 Hz, 2H), 1.84–1.73 (m, 2H), 1.32–1.23 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.2 (C), 166.9 (C), 166.4 (C), 157.8 (C) 145.1 (C), 144.8 (C), 133.5 (CH), 130.2 (CH), 125.0 (C), 121.6 (CH), 109.6 (C), 107.9 (C), 107.2 (CH), 103.7 (C), 102.4 (CH), 97.5 (CH), 90.5 (CH), 48.7 (CH_2), 33.9 (CH_2), 19.3 (CH_2), 13.6 (CH_3); LRMS (ESI+) m/z (%) 366 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{21}H_{20}NO_5$ 366.1336, found 366.1340.

*4.1.4.3. 2-(N-Butyl-5-hydroxyindol-3-ylmethylene)-4,6-dihydroxybenzofuran-3(2H)-one (**42**).* The crude product was prepared according to procedure **C** starting from compound **38** (155 mg, 0.41 mmol). After purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1), the pure product (143 mg, 0.39 mmol, 96%) was obtained as a red powder. m.p. 251–252 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.70 (s, 2H), 9.08 (s, 1H), 7.99 (s, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.24 (s, 1H), 6.89–6.62 (m, 2H), 6.26 (s, 1H), 6.06 (s, 1H), 4.34–4.01 (m, 2H), 1.86–1.60 (m, 2H), 1.38–1.17 (m, 2H), 0.96–0.78 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 178.2 (C), 166.8 (C), 166.3 (C), 157.8 (C), 152.4 (C), 144.6 (C), 133.3 (CH), 130.5 (CH), 128.2 (CH), 112.5 (C),s 111.3 (C), 106.6 (CH), 103.8 (C), 103.2 (C), 102.7 (CH), 97.4 (CH), 90.4 (CH), 46.0 (CH_2), 31.8 (CH_2), 19.5 (CH_2), 13.6 (CH_3); LRMS (ESI+) m/z (%) 366 (100) $[M+H]^+$; HRMS (ESI+) m/z calc. for $C_{21}H_{20}NO_5$ 366.1336, found 366.1340.

4.2. 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 code 2dxs) [31]. The protein structure was extracted and water molecules were deleted. The complexed ligand was used to define the binding site of docking and was successfully docked in a similar conformation in the pocket, using the same protocol as for aurones. The genetic algorithms of Autodock4 docking software 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, 100 poses were generated by Autodock, clustered according to their spatial proximity and calculated free energy of binding. The pictures were built with Pymol software [32].

4.3. Biology

4.3.1. 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, CA). The columns were washed with a buffer containing 50 mM NaH_2PO_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_2PO_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%.

4.3.2. 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, U.K.) and ATP, measured with an intercalating agent (SYBR Green, Applied Biosystems), as previously described [33]. Each experiment was performed in triplicate.

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Appendix A. Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra) related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.005.

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