Design, Synthesis and Biological Evaluation of New Embelin Derivatives as CK2 Inhibitors

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Abstract

A new series of furan embelin derivatives was synthesized and characterized as ATP-competitive CK2 inhibitors. The new compounds were efficiently synthesized using a multicomponent approach from embelin (**1**), aldehydes and isonitriles through a Knoevenagel condensation/ Michael addition/ heterocyclization. Several compounds with inhibitory activities in the low micromolar or even submicromolar were identified. The most active derivative was compound **4l** (2-(*tert*-butylamino)-3-(furan-3-yl)-5-hydroxy-6-undecylbenzofuran-4,7-dione) with an IC_{50} value of 0.63 μM. It turned out to be an ATP competitive CK2 inhibitor with a K_i value determined to be 0.48 μM. Docking studies allowed the identification of key ligand-CK2 interactions, which could help to further optimize this family of compounds as CK2 inhibitors.

1-Introduction

Casein kinase II (CK2) it has become one of the most studied protein kinases due to its constitutive activity, ubiquity, and pleiotropy [1]. CK2 typically forms tetrameric complexes consisting of two catalytic α subunits (i.e. α or α′) and two regulatory β subunits in a variety of different combinations [2]. Experimental studies have provided evidence that CK2 plays a role in the pathogenesis of cancer [3-7]. Thus, when CK2 is overexpressed in mice acts as an oncogene [4, 6-8].

Many cellular processes, which are deregulated in cancer cells, can be regulated by CK2. In this sense, CK2 increases cell growth [3], cell proliferation [9-10], cell survival [11-12], cellular transformation [6,7], promotes angiogenesis [13,14] and changes cell morphology [15,16]. All these properties make the protein kinase CK2 an attractive therapeutic target to discover new antitumor agents [17-19]. So far, only two CK2 inhibitors, CIGB-300 and CX-4945 have entered clinical trials [20]. CIGB-300 is a peptidic inhibitor that binds to the phospho-acceptor domain of CK2 substrates, thus impairing the correct phosphorylation by the enzyme, whereas CX-4945 is an ATP competitive CK2 inhibitor. Among the CK2 inhibitors some quinonic compounds have been identified such as the anthraquinones, emodin, 1,8-dihydroxy-4-nitroanthraquinone (HNA) and quinalizarin, and the naphthoquinone THN (2-(3,4-dihydroxyphenyl)-5,7-dihydroxynaphthalene-1,4-dione) [21]. In a previous work, a new family of 2-amino-4-phenyl-6-hydroxy-7-alkyl-pyranobenzoquinones as ATP-competitive CK2 inhibitors (Fig. 1a) was synthesized [22]. SAR studies of this type of compounds revealed that a) the existence of a phenyl or heteroaromatic group at the dihydropyran ring is necessary for the activity b) the presence of a C-11 alkyl chain is important since shortening of this side chain produces a drastic loss of activity c) the free hydroxyl is necessary d) a NH group as an hydrogen bond donor is key for the

activity. Taking into account the aforementioned, in the present work we design new derivatives. So we decided to replace the dihydropyran ring with a furan ring, which gives a greater planarity to the compounds. Aromatic rings as well as substituted amino groups could also be attached to the furan ring (Fig. 1b). The designed compounds in a flexible docking simulation showed similar poses and similar interactions into the ATP-binding site than those present in the previous dihydropyranbenzoquinones studied.

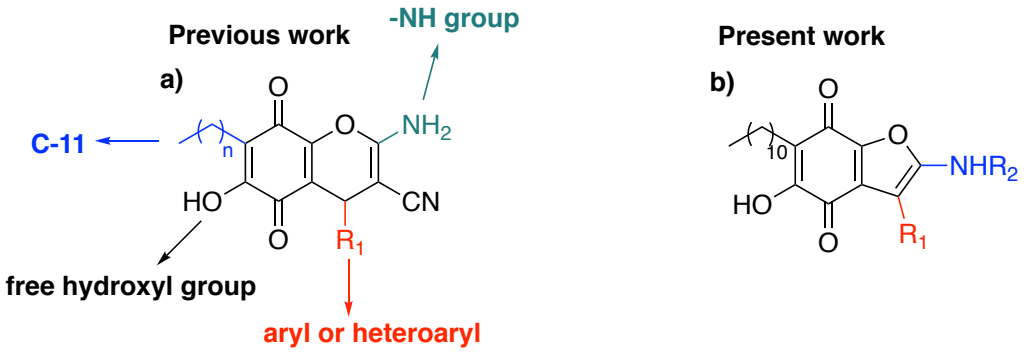
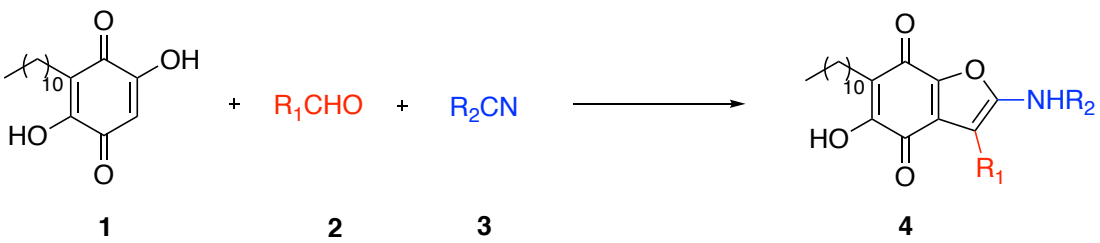


Fig. 1. a) Structure and SAR of previously synthesized benzoquinones as CK2 inhibitors and b) designed novel benzoquinones.

2-Results and discussion

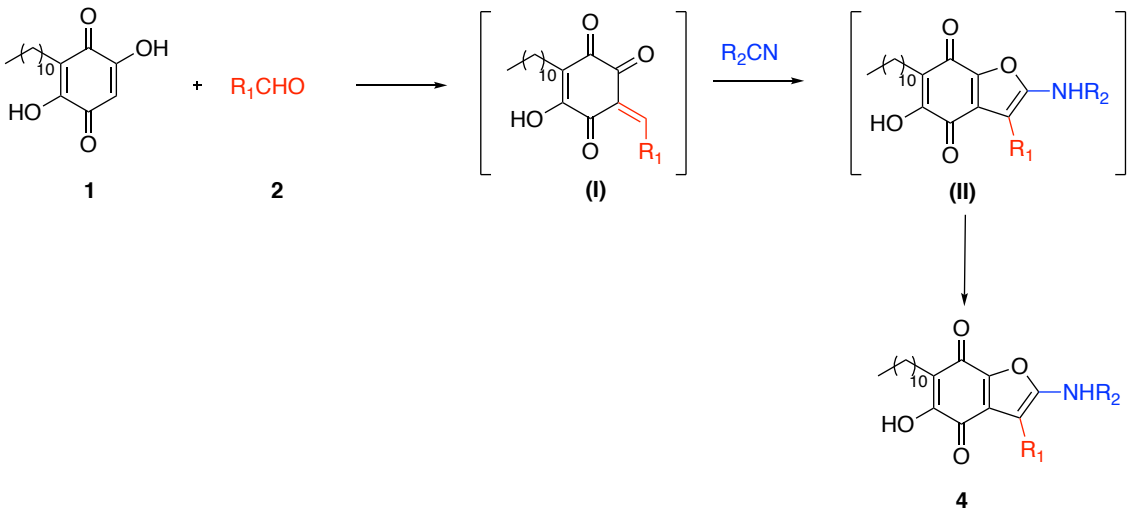
2.1 Chemistry

The designed compounds could be obtained from a multicomponent reaction involving the natural benzoquinone embeline (**1**), aldehydes (**2**) and isonitriles (**3**) (Scheme 1) [23].



Scheme 1. Synthesis of the designed furanbenzoquinones

The synthesis of furan-1,4-benzoquinone derivatives can be rationalized by the Knoevenagel condensation of embeline (**1**) and an aldehyde to yield a conjugated electron-deficient enone (**I**). Next a plausible [4 + 1] cycloaddition reaction between the isocyanide and the adduct (**I**) affords an iminolactone intermediate (**II**). The posterior isomerization of iminolactone (**II**) leads to the formation of the corresponding furan embelin derivative (Scheme 2).



Scheme 2. Plausible formation of furan embelin derivatives

The reaction was optimized using embeline (**1**), *t*-butylisonitrile and *p*-nitrobenzaldehyde as a model. Thus different organocatalysts to favour the Knoevenagel condensation (EDDA, Et_3N, proline), different ratio of reagents, solvents (DCE, EtOH, C_7H_8, CH_3CN), conventional heating and microwave irradiation were employed (Table 1). When the reaction was carried out under reflux with DCE, the desired product was isolated in low yield (7%, entry 1). The use of MW irradiation at 150ºC for 30 min increased the yield (65%, entry 2). The use of other solvents such as EtOH, C_7H_8 or CH_3CN did not improve the yield (entries 3-5). When the reaction mixture was irradiated at 180ºC a 70% yield was obtained (entry 6). Neither longer

reaction times nor the use of Et_3N or proline as catalyst led to improved yields. Therefore, the best yield was obtained when EDDA 10 mol% and dichloroethane were used under microwave irradiation at 180ºC for 15 min (70%).

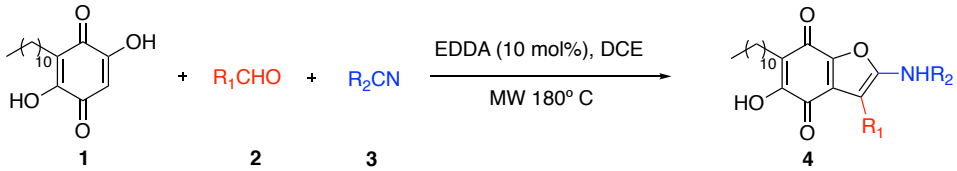
Table 1. Optimization of the reaction conditions

Entry	Ratio 1:2:3	Conditions	yield[a] (%)
1	1:1:1	10 mol% EDDA, DCE, reflux, 24 h	7
2	1:1:1	10 mol% EDDA, DCE, MW, 150º C, 30 min	65
3	1:1:1	10 mol% EDDA, EtOH, MW, 150º C, 30 min	54
4	1:1:1	10 mol% EDDA, C_7H_8, MW, 150º C, 30 min	67
5	1:1:1	10 mol% EDDA, CH_3CN, MW, 150º C, 30 min	35
6	1:1:1	10 mol% EDDA, DCE, MW, 180º C, 15 min	70
7	1:1:1	10 mol% EDDA, DCE, MW, 180º C, 20 min	68
8	1:1:2	10 mol% EDDA, DCE, MW, 180º C, 15 min	24
9	1:1:1	10 mol% Et_3N, DCE, MW, 180º C, 15 min	46
10	1:1:1	10 mol% L-proline, DCE, MW, 180º C, 15 min	48

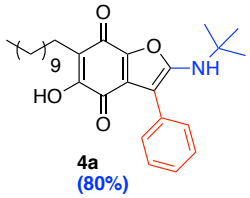
[a] Isolated yield

With the optimized protocol in hand, the scope of this multicomponent reaction was then determined through the variation of isonitriles and aldehydes (Table 2). Diversely substituted furan embelin derivatives could be prepared in moderate to good yields. As a general trend, the reaction works with a large variety of aryl-substituted aldehydes with electron-withdrawing and electron-donating groups, heteroaromatic aldehydes and aliphatic aldehydes. Regarding the isonitrile component, the best yields were achieved with the most nucleophilic *tert*-butylisocyanide.

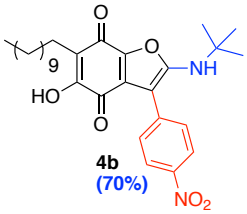
Table 2. Structures and yields of furan embelin adducts



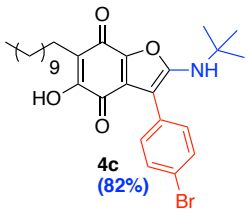
1 2 3 4



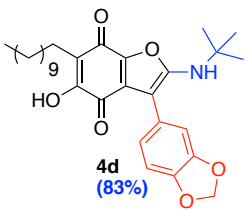
4a (80%)



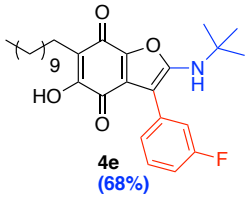
4b (70%)



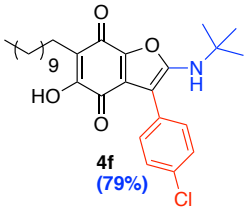
4c (82%)



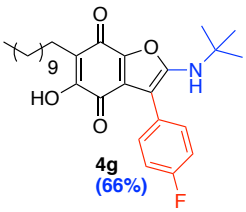
4d (83%)



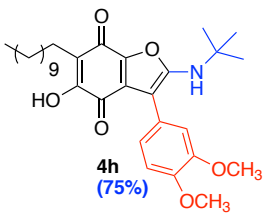
4e (68%)



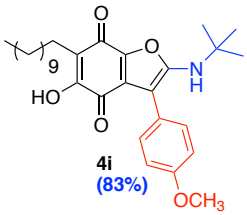
4f (79%)



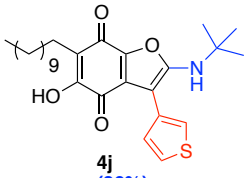
4g (66%)



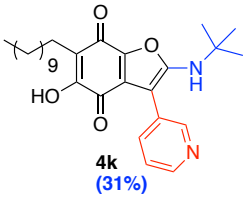
4h (75%)



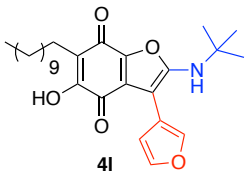
4i (83%)



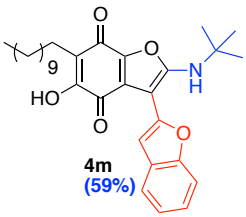
4j (36%)



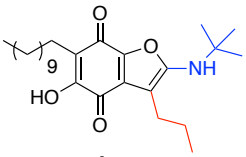
4k (31%)



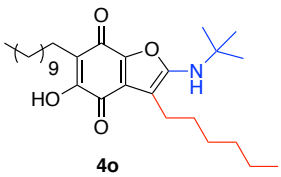
4l (60%)



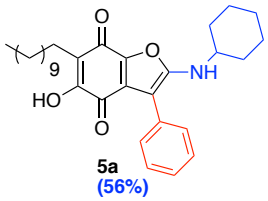
4m (59%)



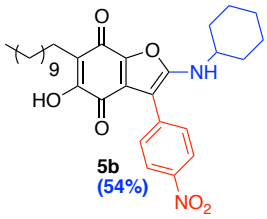
4n (69%)



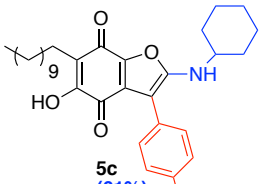
4o (64%)



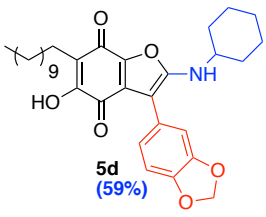
5a
(56%)



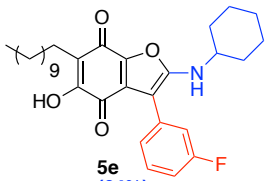
5b
(54%)



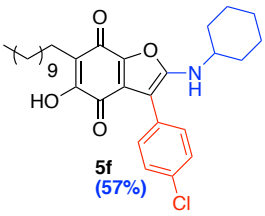
5c
(61%)



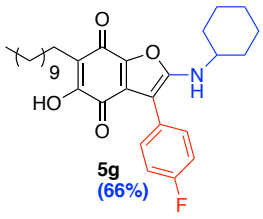
5d
(59%)



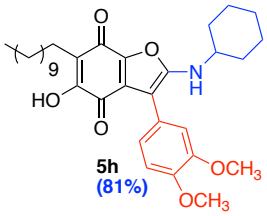
5e
(64%)



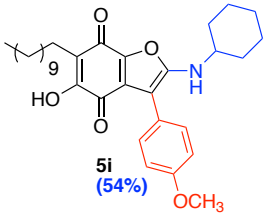
5f
(57%)



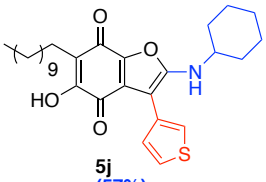
5g
(66%)



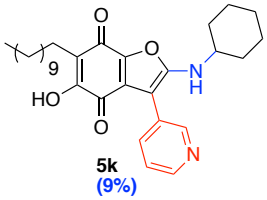
5h
(81%)



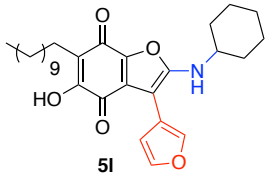
5i
(54%)



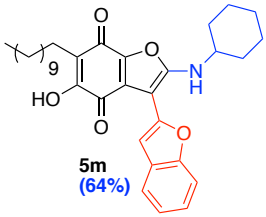
5j
(57%)



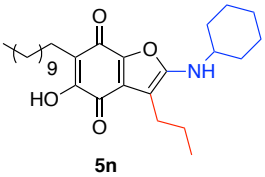
5k
(9%)



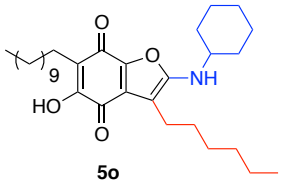
5l
(40%)



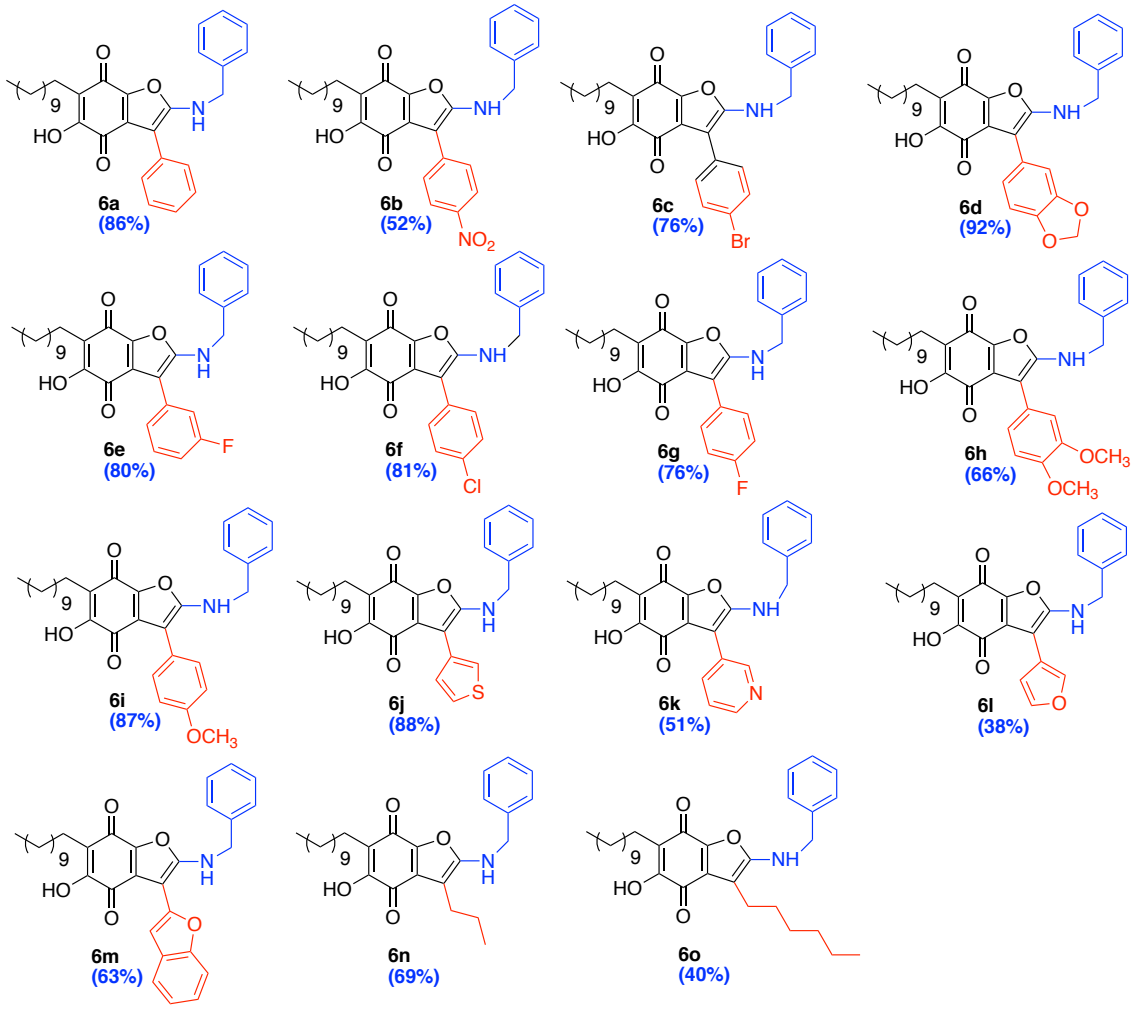
5m
(64%)



5n
(51%)



5o
(40%)



The obtained furan adducts were assayed for their inhibitory activity towards the human CK2 holoenzyme. The percent inhibition of CK2 activity was determined for each compound at a fixed concentration of 10 μM using CK2 holoenzyme and following the procedure described earlier [24]. For the best compounds producing at least 70% inhibition at 10 μM, the effect of different concentrations was analyzed in order to determine the IC_{50} value. The results obtained thereby are shown in Table 3.

Table 3. CK2 inhibitory activity of the tested furanbenzoquinones together with emodin as reference compound.

Compound	Inhibition (%)	IC_{50} ± SD (μM)
4a	65	nd (+)
4b	28	nd (+)
4c	66	nd (+)
4d	32	nd (+)
4e	9	nd (+)
4f	28	nd (+)
4g	84	2.09 ± 0.20 (++)
4h	62	nd (+)
4i	61	nd (+)
4j	79	2.33 ± 0.02 (++)
4k	52	nd (+)
4l	84	0.63 ± 0.15 (+++)
4m	51	nd (+)
4n	77	1.62 ±0.02 (++)
4o	25	nd (+)
5a	70	6.52 ± 0.06 (++)
5b	59	nd (+)
5c	63	nd (+)
5d	51	nd (+)
5e	44	nd (+)
5f	65	nd (+)
5g	70	8.36 ± 0.40 (++)
5h	53	nd (+)
5i	59	nd (+)
5j	59	nd (+)
5k	71	0.97± 0.14 (+++)
5l	92	0.98 ± 0.10 (+++)
5m	55	nd (+)
5n	73	6.10 ± 0.30 (++)
5o	71	4.57 ± 0.40 (++)
6a	47	nd (+)
6b	34	nd (+)
6c	20	nd (+)
6d	70	1.21 ± 0.09
6e	50	nd (+)
6f	65	nd (+)
6g	42	nd (+)
6h	71	1.68 ± 0.06
6i	37	nd (+)
6j	46	nd (+)
6k	25	nd (+)
6l	90	0.78 ± 0.22 (+++)
6m	43	nd (+)
6n	78	1.37 ± 0.02 (++)
6o	60	nd (+)
emodin	96	0.60 ± 0.22

nd: not determined; (+) inactive; (++) moderately active; (+++) highly active

Compounds **4a-4o**, **5a-5o** and **6a-6o** were classified by their activity as highly active (IC_{50} <1 μM, +++), moderately active (1 μM < IC_{50} < 10 μM, ++), or inactive (10 μM > IC50, +).

The best inhibitors of CK2 turned out to be compounds **4l**, **5l** and **6l**, obtained from 3-furaldehyde, with IC_{50} values in the submicromolar range, in the same order of magnitude as the reference compound emodin. With the same aryl substituent at the fused furan ring, the benzyl derivatives produced the highest activities in most cases (i.e. **6d** *vs* **5d** and **4d**; **6h** *vs* **5h** and **4h; 6n** *vs* **5n** and **4n**).

Based on the effects of the furanbenzoquinones as synthesized on human CK2 holoenzyme, we used a docking computational approach in the next step to assess the potential interactions of different furanembelin derivatives with the narrow ATP-binding site of CK2. The binding pattern of the most actives compounds was analyzed by flexible molecular docking in order to propose the mode of action of the significant inhibitory effects of derivatives **4l** and **6l**. Thus, a molecular docking study was carried out using the Glide software [25] on reported crystal structure of human protein kinase CK2 alpha subunit in complex with the inhibitor CX-4945 (PDB 3PE1). According to the hypothetical model for the binding mode of the protein kinase inhibitors proposed by Traxler and Furet [26], the ATP-binding site can be divided into five regions, three hydrophobic regions corresponding to the adenine region and the hydrophobic regions I and II and also two hydrophilic regions that correspond one of them to the sugar pocket and another one to the phosphate binding region. ATP competitive inhibitors of kinases, typically form one to three hydrogen bonds with the backbone of residues of the hinge region. These hydrogen bonds mimic those which are normally formed by the amino group of ATP [27]. These features can be also applied to the CK2 protein. Hence, in order to explore the binding mode of **4l** and **6l**, these compounds have been inserted into

the narrow ATP binding site of CK2. An analysis of the docking results showed that the compounds fit very well and, as shown in Fig.2, the active site is fully occupied by the compound **4l**, the aliphatic chain was located at the edge of the pocket and established hydrophobic interactions with the hydrophobic region I, in the deepest part of the cavity and with the hydrophobic region II at the entrance of the cavity as well as the adenine region. The compound **4l** established hydrophobic interactions and van der Waals contacts involving the hydrophobic surface of the CK2 binding site formed by side chains of Val 53, Val 66, Val 116, Met 163 (adenine region), Leu 45, His 115 (hydrophobic region II), Phe 113, Ile 95 and Ile 174 (hydrophobic region I). Another distinctive feature of CK2 is the presence of a salt bridge between Lys68 and Glu81, this region is one of the most important polar anchoring points for CK2 inhibitors. In this sense, one hydrogen bond was observed between the hydroxyl group of **4l** and Lys 68. In the predicted pose of **4l** another representative interaction was a π-π stacking between the furan ring with the side chain of His 160. The high activity achieved by compound **4l** can be also explained on the basis of the existence of one hydrogen bond interactions between the hydroxyl group and Lys 68, and finally the alkyl chain of the compound **4l** and the interactions established with the side chains residues on the hydrophobic surface of the CK2 binding site, reinforce the key role played by the C-11 chain and the apolar forces involving van der Waals contacts interactions between the hydrophobic region I, the adenine region and the hydrophobic region II driving the orientation of the compounds into the ATP binding site as it was reported previously [22].

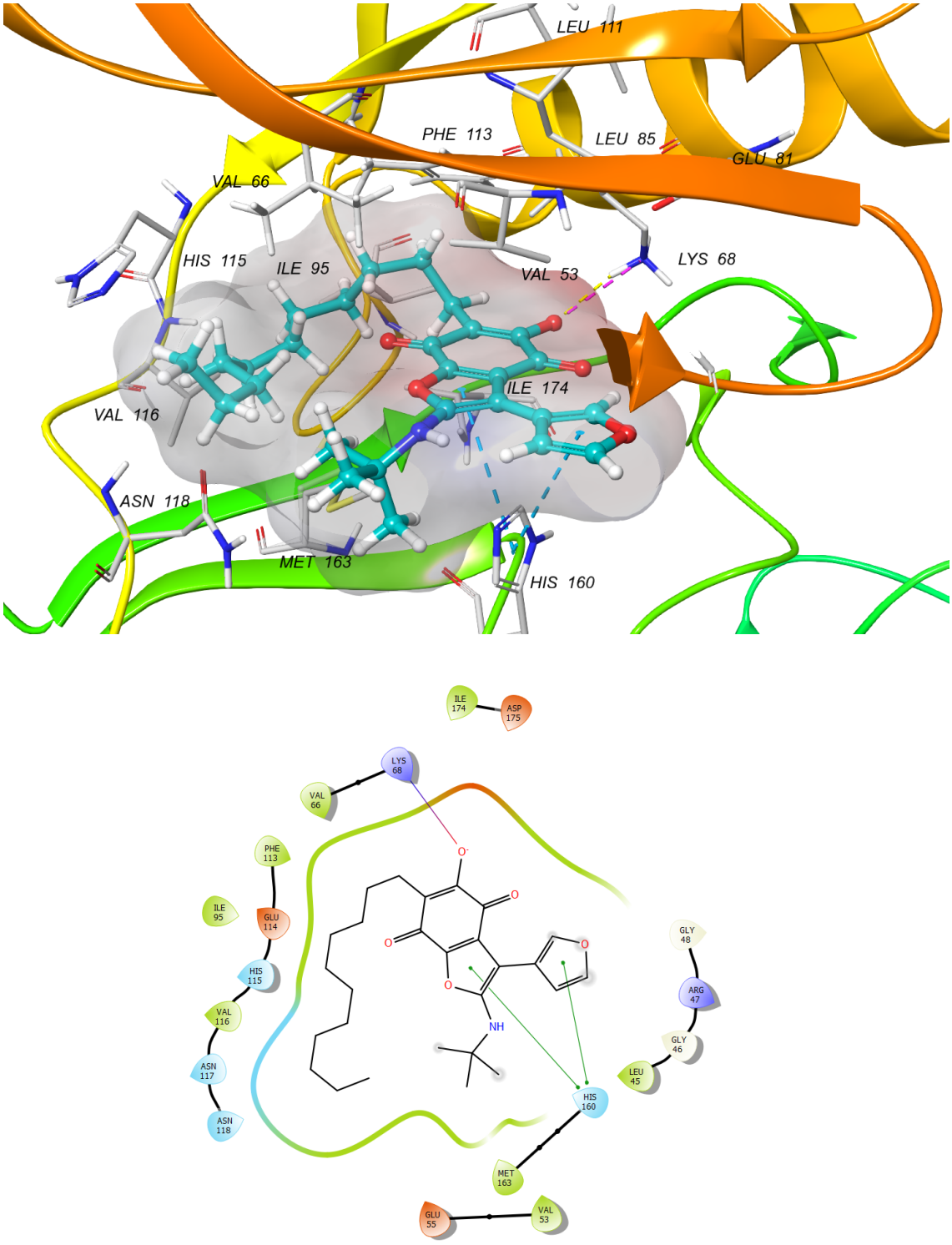


Fig. 2. 3D and 2D representation of the binding mode of **4l** with CK2 (PDB 3PE1).

An analysis of the docking results for compound **6l** showed that the aliphatic chain was located outside the active site, therefore, the hydrophobic surface of the CK2 binding site is not fully occupied. As previously mentioned, according to the

pharmacophore model for protein kinase inhibitors that bind principally to the hydrophobic adenine region, they constitute the vast majority of the currently available protein kinase ATP-competitive inhibitors. Nevertheless, some protein kinase inhibitors usually show ATP-competitive behavior and preferentially have contacts to the hinge region and they present hydrophobic interactions in a pocket adjacent to the ATP-binding site. In this sense, the activity of this compound on the CK2 protein could be explained by the presence of two hydrogen bonds, one of them between the hydroxyl group of compound **6l** and Lys 68 and another hydrogen bond between the furan ring and Val 116 from the hinge region. The structural portion constituted by a benzyl group established Van der Waals interactions with the side chains of Leu 45, Met 163 and Asn 118. Finally, as shown in Fig. 3 the long lipophilic chain of compound **6l** make hydrophobic contacts in adjacent areas to the active site with Asp 175, Lys 158, Asp 156, Leu 178 and Ala 193.

The predicted binding modes and the presence of different substituents on the amino group of the furanbenzoquinones suggested that the best docked pose for each ligand and the binding mode of these compounds (**4l**, **5l**, and **6l**) to the CK2 protein was driven depending on the degree of steric interactions between the *tert*-butyl group (89.56 Å), benzyl group (115.77 Å) or cyclohexyl group (110.65 Å) and the corresponding aliphatic chain. This fact reinforces the key role that the C-11 plays driving the properly orientation of the compounds to interact favorably within the lining of the ATP-binding site.

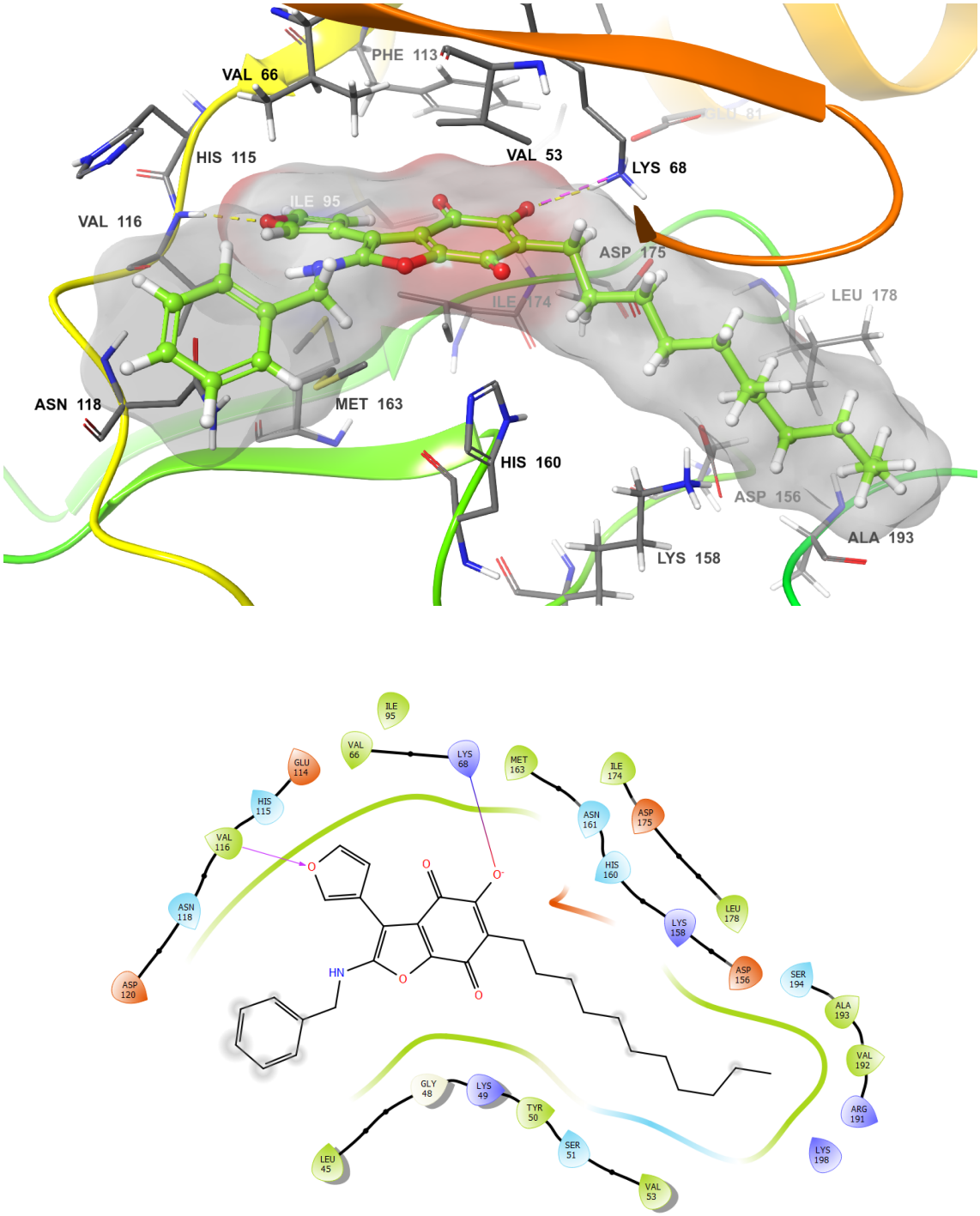


Fig. 3. 3D and 2D representation of the binding mode of **6l** with CK2 (PDB 3PE1).

The mode of action of this set of compounds was also ratified by determining the IC_{50} values of the most potent inhibitor **4l** at different ATP concentrations. Compound **4l** was selected as a representative inhibitor of this series, and the IC_{50} values were determined at eight different concentrations of compound **4l**, ranging from

0.001 to 100 μM, and repeated four times using different ATP concentrations each time. The IC_{50} values increased linearly with the ATP concentration, which is indicative of an ATP competitive mode of CK2 inhibition (Fig. 4). The K_i value of CK2 inhibition by compound **4l** was determined from the IC_{50} values obtained at various ATP concentrations. The regression line showed a Y-axis intercept at an IC_{50} value of 0.48 ± 0.07 μM (Fig. 4) and thereby defined the K_i value of compound **4l** to be at this concentration.

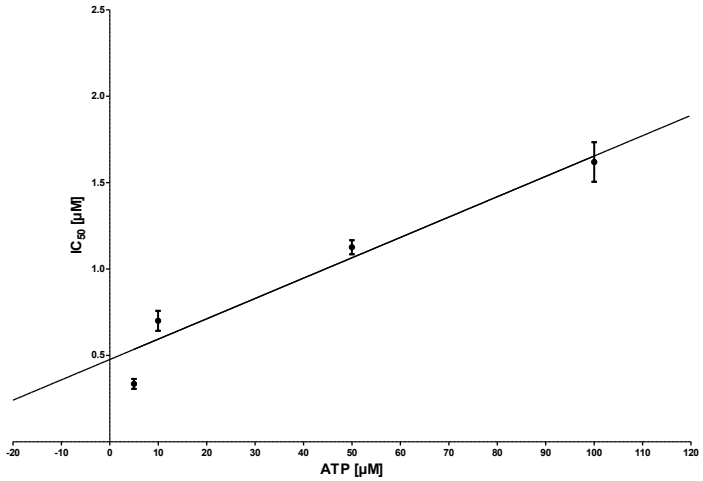


Fig. 4. ATP-competitive inhibition of human CK2 by compound **4l**. Four IC_{50} values with different ATP concentrations were determined using eight different concentration of the inhibitor, ranging from 0.001 to 100 μM and plotted against the respective ATP concentrations. Each IC_{50} value was determined 3 times independently. Mean values with corresponding standard deviation are given. The K_i value is defined as the Y-intercept and was determined to be 0.48 ± 0.07 μM (R^2= 0.90).

Compounds **4l**, **5l** and **6l**, which appeared to be the most potent CK2 inhibitors in the current study with IC_{50} values of 0.63 μM to 0.98 μM and 0.78 μM respectively, were selected to evaluate the antiproliferative effect of this group of furanbenzoquinones. MCF7 (human breast adenocarcinoma cell line) cells were treated with different concentrations of each compound, cell viability was tested applying MTT assay using the above mentioned cell line. Also the antiproliferative effect of compounds **4l**, **5I**, and **6I** was investigated with the same cells. For this purpose, a commercially available EdU-click assay was applied, resulting in the coupling of a

TAMRA fluorophore into the nucleic acid of cells performing DNA replication and hence preparing cell division. Proliferating cells can be recognized by a violet fluorescence of their nuclei. The number of proliferating cells was determined after treatment with compounds **4l, 5I**, or **6I** in various concentrations and was set into relation to the total number of cells obtained with the 1% DMSO control. In both experiments only minor effects were seen with all compounds even at concentration of 100 μM (see Supplementary Material).

Conclusion

A series of furan embelin derivatives were designed and synthesized as CK2 inhibitors from a multicomponent reaction using the natural benzoquinone embelin (**1**), aldehydes and isonitriles. Some of them inhibited CK2 activity at the micromolar or submicromolar levels. The mode of action was established on the basis of ATP competitive assays. For the most active compounds the key interactions into the ATP binding site of CK2 were analyzed by docking studies, which reinforced the role of the C-11 alkyl chain in the orientation of the compounds to interact favorably. Thus in compounds having the N-*t*-butyl group, the aliphatic chain occupies the edge of the binding site, while with N-cyclohexyl and N-benzyl group the alkyl chain is located outside of the ATP-binding site. In all these cases the key interaction between the hydroxyl group of the quinone moiety and Lys 68 is present. Based on these results, the design and synthesis of new embelin derivatives will be considered in order to obtain more potent CK2 inhibitors.

Experimental section

NMR spectra were recorded in $CDCl_3$ at 500 or 600 MHz for ^{1}H NMR and 125 or 150 MHz for ^{13}C NMR. Chemical shifts are given in (δ) parts per million and coupling

constants (*J*) in hertz (Hz). ^{1}H and ^{13}C spectra were referenced using the solvent signal as internal standard. Melting points were taken on a capillary melting point apparatus and are uncorrected. HREIMS were recorded using a high-resolution magnetic trisector (EBE) mass analyzer. Analytical thin-layer chromatography plates used were Polygram-Sil G/UV254. Preparative thin-layer chromatography was carried out with Analtech silica gel GF plates (20 x 20 cm, 1000 Microns) using appropriate mixtures of ethyl acetate and hexanes. All compounds were named using the ACD40 Name-Pro program, which is based on IUPAC rules. The embelin (**1**) used in the reactions was obtained from *Oxalis erythrorhiza Gillies ex Hook. & Arn.* following the procedure described in reference [28].

4.2 General Procedures for the preparation of dihydropyran embeline derivatives

To a mixture of 0.1 mmol of the corresponding isocyanide, 0.1 mmol of the corresponding aldehyde, and 10 mol % of EDDA in 2 mL of DCE, were added 30 mg of embelin (0.1 mmol). The reaction mixture was stirred and irradiated for 15 min at 180ºC. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC to yield the corresponding furan derivative.

2-(*tert*-butylamino)-5-hydroxy-3-phenyl-6-undecylbenzofuran-4,7-dione (4a)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 10.46 μL of benzaldehyde (0.102 mmol), 11.7 μL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 38.2 mg (80%) of **4a** as a dark blue oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.3 Hz, 3H), 1.25 (bs, 16H), 1.42 (s, 9H), 1.50 (m, 2H), 2.47 (t, *J*= 7.5 Hz, 2H), 4.64 (bs, 1H), 6.86 (bs, 1H), 7.33 (m, 1H), 7.43 (m, 4H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 22.8

(CH_3), 27.9 (CH_2), 28.3 (CH_3), 28.4 (CH_3 x 2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 30.1 (CH_2), 31.9 (CH_2), 52.6 (C), 99.6 (CH), 118.7 (C), 119.1 (C), 125.5 (CH), 128.7 (CH), 130.2 (C), 134.1(CH), 141.5 (C), 150.3 (C), 157.7 (C), 174.3 (C), 179.6 (C).EIMS *m/z* (%): 465 ($[M]^+$, 41), 409 (100), 270 (19), 269 (27), 154 (8). HREIMS: 465.2878 (calcd for $C_{29}H_{39}O_4N$, $[M]^+$ 465.2879).

2-(*tert*-butylamino)-5-hydroxy-3-(4-nitrophenyl)-6-undecylbenzofuran-4,7-dione (4b)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.4mg of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 36.6 mg (70%) of **4b** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.5 Hz, 3H), 1.25 (bs, 16H), 1.46 (s, 9H), 1.52 (m, 2H), 2.47 (t, J= 7.4 Hz, 2H), 4.74 (s, 1H), 7.62 (d, *J*= 8.7 Hz, 2H), 8.27 (d, *J*= 8.7 Hz, 2H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.6 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 30.1 (CH_3 x 3), 31.9 (CH_2), 54.3 (C), 96.9 (C), 119.2 (C), 123.2 (C), 124.1 (CH x 2), 129.2 (CH x 2), 137.7 (C), 144.2 (C), 146.3 (C), 150.5 (C), 157.8 (C), 174.5 (C), 179.4 (C).EIMS m/z (%): 510 ($[M]^+$, 1), 454 (100), 315 (21), 314 (30), 277 (10).HREIMS: 510.2722 (calcd for $C_{29}H_{38}O_6N_2$, $[M]^+$ 510.2730).

3-(4-bromophenyl)-2-(*tert*-butylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4c)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 18.87 mg of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The

solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 45.3 mg (82%) of **4c** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.42 (s, 9H), 2.46 (t, *J*= 7.6 Hz, 2H), 4.54 (bs, 1H), 6.85 (bs, 1H), 7.31 (d, *J*= 8.4 Hz, 2H), 7.56 (d, *J*= 8.5 Hz, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.2 (CH_3), 22.5 (CH_2), 27.9 (CH_2), 28.2 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 30.1 (CH_3 x 3), 31.9 (CH_2), 53.9 (C), 60.4 (C), 94.8 (C), 119.2 (C), 121.4 (C), 123.5 (C), 130.5 (CH x 2), 132.1 (CH x 2), 143.6 (C), 150.3 (C), 157.5 (C), 174.3 (C), 179.5 (C).EIMS m/z (%): 545 ($[M]^+$, 37), 490 (28), 489 (100), 488 (29), 487 (98), 349 (19), 348 (32), 346 (30), 57 (40).HREIMS: 545.1978 (calcd for $C_{29}H_{38}O_4N^{81}Br$, $[M]^+$ 545.1964) and 543.1966 (calcd for $C_{29}H_{38}O_4N^{79}Br$, $[M]^+$ 543.1984).

3-(benzo[d][1,3]dioxol-5-yl)-2-(*tert*-butylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4d)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.47mg of aldehyde (0.102 mmol), 11.7 μL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 43.1 mg (83%) of **4d** as a dark green oil.^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 7.1 Hz, 3H), 1.25 (bs, 16H), 1.50 (m, 2H), 1.41 (s, 9H), 1.46 (m, 2H), 2.46 (t, *J*= 7.4 Hz, 2H), 4.54 (s, 1H), 6.01 (s, 2H), 6.83 (s, 1H), 6.86 (d, *J*= 1.2 Hz, 1H), 6.91 (d. *J*= 1.2 Hz, 1H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 3), 30.1 (CH_3 x 3), 31.9 (CH_2), 53.9 (CH_2), 99.5 (C), 101.3 (CH_2), 108.7 (CH), 109.6 (CH), 119.1 (C), 122.2 (CH), 123.7 (C), 143.4 (C), 147.2 (C), 148.2

(C), 150.3 (C), 157.7 (C), 174.2 (C), 179.7 (C). EIMS m/z (%): 509 ($[M]^+$, 1), 453 (100), 314 (17), 313 (27). HREIMS: 509.2778 (calcd for $C_{30}H_{39}O_6N$, $[M]^+$ 509.2777).

2-(*tert*-butylamino)-3-(3-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4e)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 11.05 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 33.7 mg (68%) of **4e** as a dark blue oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 7.1 Hz, 3H), 1.25 (bs, 16H), 1.43 (s, 9H), 1.50 (m, 2H), 2.46 (t, J= 7.2 Hz, 2H), 4.62 (s, 1H), 6.86 (s, 1H), 7.02 (dt, *J*= 8.5, 1.8 Hz, 1H), 7.14 (dd, *J*= 10.1, 2.0 Hz, 1H), 7.20 (d, *J*= 8.0 Hz, 1H), 7.40 (dd, *J*= 9.8, 1.8 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 30.1 (CH_3 x 3), 31.9 (CH_2), 54.0 (C), 98.2 (C), 114.4 (CH, J_{CF}= 20.9 Hz), 115.8 (CH, J_{CF}= 22.1 Hz), 119.1 (C), 123.6 (C), 124.3 (CH, J_{CF}= 1.7 Hz), 130.4 (CH, J_{CF}= 8.9 Hz), 132.5 (C, J_{CF}= 8.3 Hz), 143.7 (C), 150.4 (C), 157.6 (C), 163.0 (CH, J_{CF}= 245.2 Hz), 174.4 (C), 179.5 (C). EIMS m/z (%): 483 ($[M]^+$, 1), 427 (100), 294 (38), 288 (22), 287 (33). HREIMS: 483.2763 (calcd for $C_{29}H_{38}O_4NF$, $[M]^+$ 483.2785).

2-(tert-butylamino)-3-(4-chlorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4f)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 14.78 mg of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-

TLC with 20% hexanes/EtOAc to yield 40.1 mg (79%) of **4a** as a dark green oil.[1]H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.9 Hz, 3H), 1.25 (bs, 14H), 1.39 (m, 2H), 1.42 (s, 9H), 1.49 (m, 2H), 2.46 (t, *J*= 7.6 Hz, 2H), 7.36 (d, *J*= 8.7 Hz, 2H), 7.41 (d, *J*= 8.7 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 27.9 (CH_2), 28.3 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 30.1 (CH_3 x 3), 31.9 (CH_2), 54.0 (C), 98.4 (C), 119.2 (C), 123.7 (C), 127.0 (C), 129.1 (CH x 2), 130.2 (CH x 2), 133.3 (C), 143.7 (C), 150.4 (C), 157.6 (C), 174.3 (C), 179.6 (C). EIMS *m/z* (%): 499 ([M]$^+$, 30), 445 (38), 443 (100), 303 (28), 302 (37), 57 (27). HREIMS: 499.2493 (calcd for $C_{29}H_{38}O_4NCl$, [M]$^+$ 499.2489).

2-(*tert*-butylamino)-3-(4-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4g)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 11.70 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 32.7 mg (66%) of **4g** as a dark blue oil.[1]H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.8 Hz, 3H), 1.25 (bs, 16H), 1.42 (s, 9H), 1.49 (m, 2H), 2.46 (t, *J*= 7.4 Hz, 2H), 4.49 (bs, 1H), 6.83 (bs, 1H), 7.13 (t, *J*= 8.6 Hz, 2H), 7.40 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 39.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2), 30.1 (CH_3 x 3), 31.9 (CH_2), 53.9 (C), 98.8 (C), 115.9 (CH x 2, J_{CF}= 21.6 Hz), 119.2 (C), 124.0 (C), 126.7 (C), 130.7 (CH x 2, J_{CF}= 8.3 Hz), 143.6 (C), 150.3 (C), 157.5 (C), 162.1 (C, J_{CF}= 246.5 Hz), 174.3 (C), 179.6 (C). EIMS m/z (%): 483 ([M]$^+$, 1), 427 (100), 294 (38), 288 (7), 287 (11). HREIMS: 483.2771 (calcd for $C_{29}H_{38}O_4NF$, [M]$^+$ 483.2785).

2-(*tert*-butylamino)-3-(3,4-dimethoxyphenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4h)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 16.95mg of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 39.8 mg (75%) of **4h** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.7 Hz, 3H), 1.25 (bs, 16H), 1.45 (bs, 9H), 1.48 (m, 2H), 2.47 (t, *J*= 7.6 Hz, 2H), 3.91 (s, 3H), 3.93 (s, 3H), 4.62 (bs, 1H), 6.87 (bs, 1H), 6.95 (m, 2H), 7.02 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7 (CH_2), 28.3 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 30.6 (CH_3 x 3), 31.9 (CH_2), 53.9 (C), 55.9 (CH_3), 56.0 (CH_3), 99.9 (C), 111.4 (CH), 112.7 (CH), 119.1 (C), 120.7 (CH), 122.7 (C), 123.8 (C), 143.4 (C), 148.5 (C), 149.1 (C), 150.3 (C), 157.7 (C), 174.2 (C), 179.7 (C). EIMS *m/z* (%): 525 ($[M]^+$, 33), 469 (60), 300 (37), 285 (86), 256 (31), 239 (70), 197 (36), 165 (30), 146 (32), 129 (61), 115 (33), 85 (33), 73 (100), 60 (85), 57 (90), 55 (84). HREIMS: 525.3063 (calcd for $C_{31}H_{43}O_6N$, $[M]^+$ 525.3090).

2-(*tert*-butylamino)-5-hydroxy-3-(4-methoxyphenyl)-6-undecylbenzofuran-4,7-dione (4i)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 12.72 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 41.7 mg (83%) of **4i** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.8 Hz, 3H), 1.25 (bs, 18H), 1.41 (s, 9H), 2.46 (t,

J= 7.6 Hz, 2H), 3.85 (s, 3H), 4.51 (bs, 1H), 6.82 (bs, 1H), 6.97 (d, *J*= 8.7 Hz, 2H), 7.35 (d, *J*= 8.7 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 30.2 (CH_3 x 3), 31.9 (CH_2), 53.9 (C), 55.3 (CH_3), 99.8 (C), 114.3 (CH x 2), 119.2 (C), 122.2 (C), 124.0 (C), 130.1 (CH x 2), 132.0 (C), 143.4 (C), 150.3 (C), 157.7 (C), 159.0 (C), 174.1 (C), EIMS *m/z* (%): 495 ([M]$^+$, 42), 439 (100), 299 (50), 285 (65), 239 (47), 129 (42), 73 (72), 60 (57). HREIMS: 495.2987 (calcd for $C_{30}H_{41}O_5N$, [M]$^+$ 495.2985).

2-(*tert*-butylamino)-5-hydroxy-3-(thiophen-3-yl)-6-undecylbenzofuran-4,7-dione (4j)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 9.10 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 17.2 mg (36%) of **4j** as a dark blue oil.^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.4 Hz, 3H), 1.25 (bs, 16H), 1.44 (s, 9H), 1.49 (m, 2H), 2.47 (t, J= 7.4 Hz, 2H), 4.62(s, 1H), 7.33 (dd, *J*= 4.8, 0.8 Hz, 1H), 7.42 (m, 1H), 7.46 (dd, *J*= 2.8, 1.2 Hz, 1H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 30.1 (CH_3 x 3), 31.9 (CH_2), 53.9(C), 95.5 (C), 119.2 (C), 122.9 (CH), 126.1 (CH), 127.9 (CH), 128.9 (C), 130.4 (C), 143.4 (C), 150.5 (C), 157.7 (C), 174.2 (C), 179.6 (C). EIMS m/z (%): 471 ([M]$^+$, 1), 415 (100), 294 (12), 276 (21), 275 (29). HREIMS: 471.2448 (calcd for $C_{27}H_{37}O_4NS$, [M]$^+$ 471.2443).

2-(*tert*-butylamino)-5-hydroxy-3-(pyridin-3-yl)-6-undecylbenzofuran-4,7-dione (4k)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 10.00 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and

1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 14.8 mg (31%) of **4k** as a dark green oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.7 Hz, 3H), 1.25 (bs, 16H), 1.43 (s, 9H), 1.50 (m, 2H), 2.48 (t, *J*= 7.4 Hz, 2H), 4.55 (s, 1H), 7.38 (dd, *J*= 8.5, 5.5 Hz, 1H), 7.80 (td, *J*= 8.2, 1.7 Hz, 1H), 8.56 (dd, *J*= 7.4, 1.2 Hz, 1H), 8.71 (d, *J*= 1.6 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 30.1 (CH_3 x 3), 31.9 (CH_2), 54.2 (C), 95.9 (C), 119.3 (C), 123.6 (CH), 123.7 (C), 126.8 (C), 136.7 (CH), 144.1 (C), 148.3 (CH), 149.3 (CH), 150.7 (C), 157.7 (C), 174.5 (C), 179.6 (C). EIMS m/z (%): 466 ($[M]^+$, 1), 410 (100), 270 (18), 269 (27), 268 (12). HREIMS: 466.2822 (calcd for $C_{28}H_{38}O_4N_2$, $[M]^+$ 466.2832).

2-(*tert*-butylamino)-3-(furan-3-yl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (4l)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 8.78 μL of aldehyde (0.102 mmol), 11.7 μL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 27.6 mg (60%) of **4l** as a dark blue oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.2 Hz, 3H), 1.25 (bs, 16H), 1.44 (s, 9H), 1.51 (m, 2H), 2.46 (t, *J*= 8.0 Hz, 2H), 6.74 (d, *J*= 1.2 Hz, 1H), 7.51 (t, *J*= 1.5 Hz, 1H), 7.85 (d, *J*= 0.7 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.3 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.7 (CH_2), 30.1 (CH_3 x 3), 31.9 (CH_2), 54.1 (C), 92.6 (C), 109.9 (CH), 114.9 (CH), 119.2 (C), 123.6 (C), 140.8 (CH), 143.3 (CH), 150.3(C), 153.3 (C), 157.2 (C), 174.4 (C), 179.5 (C). EIMS m/z (%):

455 ([M]$^+$, 1), 400 (23), 399 (100), 315 (22), 258 (5). HREIMS: 455.2668 (calcd for $C_{27}H_{37}O_5N$, [M]$^+$ 455.2672).

2'-(*tert*-butylamino)-5'-hydroxy-6'-undecyl-[2,3'-bibenzofuran]-4',7'-dione (4m)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 12.36 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 30.4 mg (59%) of **4m** as a dark blue oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.8 Hz, 3H), 1.25 (bs, 16H), 1.55 (s, 9H), 1.49 (m, 2H), 2.48 (t, *J*= 7.6 Hz, 2H), 6.45 (bs, 1H), 6.98 (bs, 1H), 7.23 (m, 2H), 7.39 (m, 1H), 7.56 (m, 1H), 7.63 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 30.0 (CH_2), 30.0 (CH_3 x 3), 31.9 (CH_2), 54.0 (C), 89.2 (C), 103.6 (CH), 110.2 (CH), 119.3 (C), 120.9 (CH), 121.9 (C), 123.4 (CH), 123.8 (CH), 128.9 (C), 143.1 (C), 148.9 (C), 150.2 (C), 153.4 (C), 158.3 (C), 173.8 (C), 179.1 (C). EIMS *m/z* (%): 505 ([M]$^+$, 44), 449 (100), 309 (20), 308 (32), 57 (8). HREIMS: 505.2803 (calcd for $C_{31}H_{39}O_5N$, [M]$^+$ 505.2828).

2-(*tert*-butylamino)-5-hydroxy-3-propyl-6-undecylbenzofuran-4,7-dione (4n)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 9.20 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 30.2 mg (69%) of **4n** as a dark green oil.^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.5 Hz, 3H), 0.92 (t, *J*= 7.4 Hz, 3H), 1.25 (bs, 16H), 1.36 (bs, 9H), 1.45 (m, 2H), 1.56 (m, 2H), 2.44 (m, 4H), 6.84 (s, 1H).^{13}C-NMR (100

MHz, $CDCl_3$) δ 13.8 (CH_3), 13.9 (CH_3), 22.5 (CH_2), 24.9 (CH_2), 27.9 (CH_2), 28.2 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2 x 2), 29.6 (CH_2 x 3), 30.2 (CH_3 x 3), 31.9 (CH_2), 53.9 (C), 103.5 (C), 119.4 (C), 125.1 (C), 144.2 (C), 150.3 (C), 157.3 (C), 174.5 (C), 180.2 (C).EIMS m/z (%): 431 ($[M]^+$, 15), 375 (100), 346 (12), 235 (24), 234 (25), 57 (15).HREIMS: 431.3052 (calcd for $C_{26}H_{41}O_4N$, $[M]^+$ 431.3036).

2-(*tert*-butylamino)-3-hexyl-5-hydroxy-6-undecylbenzofuran-4,7-dione (4o)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 14.98 µL of aldehyde (0.102 mmol), 11.7 µL of *t*-butyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 30.7 mg (64%) of **4o** as a brown oil.^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.1 Hz, 6H), 1.25 (bs, 14H), 1.36 (s, 9H), 1.48 (m, 4H), 2.44 (t, *J*= 7.4 Hz, 4H), 3.67 (s, 1H), 6.83 (s, 1H).^{13}C-NMR (100 MHz, $CDCl_3$) δ 14.0 (CH_3), 14.1 (CH_2), 22.6 (CH_2), 22.7 (CH_2), 22.9 (CH_2), 28.5 (CH_2), 29.0 (CH_2), 29.3 (CH_2 x 2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 30.3 (CH_3 x 3), 31.6 (CH_2), 31.9 (CH_2), 53.9 (C), 103.9 (C), 119.4 (C), 125.0 (C), 144.3 (C), 150.3 (C), 157.3 (C), 174.4 (C), 180.2 (C).EIMS m/z (%): 473 ($[M]^+$, 1), 417 (100), 416 (10),346 (10), 277 (16), 276 (12). HREIMS: 473.3488 (calcd for $C_{29}H_{47}O_4N$, $[M]^+$ 473.3505).

2-(cyclohexylamino)-5-hydroxy-3-phenyl-6-undecylbenzofuran-4,7-dione (5a)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 10.46 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 28.1 mg (56%) of **5a** as a dark blue oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1Hz, 3H), 1.25 (bs, 18H), 1.37 (m, 3H),

1.50 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.03 (m, 2H), 2.47 (t, *J*= 7.6 Hz, 2H), 3.70 (m, 1H), 4.61 (d, *J*= 8.5 Hz, 1H), 6.83 (bs, 1H), 7.32(m, 1H), 7.44 (d, *J*= 4.4 Hz, 4H).^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 34.0 (CH_2), 52.5 (CH), 97.3 (C), 119.1 (C), 124.6 (C), 127.4 (CH), 128.7 (CH x 2), 128.8 (CH x 2), 130.2 (C), 142.6 (C), 150.2 (C), 157.5 (C), 174.2 (C), 179.7 (C).EIMS *m/z* (%): 491 ($[M]^+$, 100), 409 (45), 351 (53), 268 (62), 212 (13), 83 (19), 55 (58). HREIMS: 491.3072 (calcd for $C_{31}H_{41}O_4N$, $[M]^+$ 491.3036).

2-(cyclohexylamino)-5-hydroxy-3-(4-nitrophenyl)-6-undecylbenzofuran-4,7-dione (5b)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.40 mg of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.5 mg (54%) of **5b** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.2 Hz, 3H), 1.25 (bs, 18H), 1.39 (m, 3H), 1.77 (m, 2H), 1.66 (m, 1H), 1.76 (m, 2H), 2.06 (m, 2H), 2.47 (t, *J*= 7.6 Hz, 2H), 3.76 (m, 1H), 4.85 (d, *J*= 8.1 Hz, 1H), 6.88 (bs, 1H), 7.62 (d, *J*= 8.6 Hz, 2H), 8.24 (d, *J*= 8.5 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.3 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 33.9 (CH_2), 52.5 (CH), 94.8 (C), 119.2 (C), 123.9 (C), 124.1 (CHx2), 129.1 (CHx2), 137.7 (C), 143.3 (C), 146.1 (C), 150.5 (C), 157.7 (C), 174.5 (C), 179.5 (C). EIMS m/z (%): 536 ($[M]^+$, 100), 491 (61), 454 (51), 396 (70), 314 (65), 83 (33), 55 (74). HREIMS: 536.2919 (calcd for $C_{31}H_{40}O_6N_2$, $[M]^+$ 536.2886).

3-(4-bromophenyl)-2-(cyclohexylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5c)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 18.87mg of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 35.1 mg (61%) of **5c** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.38 (m, 3H), 1.48 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.02 (m, 2H), 2.46 (t, *J*= 7.4 Hz, 2H), 3.69 (m, 1H), 4.57 (d, *J*= 8.6 Hz, 1H), 6.83 (bs, 1H), 7.31 (d, *J*= 8.5 Hz, 2H), 7.54 (d, *J*= 8.3 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.3 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 33.9 (CH_2), 52.7 (CH), 94.8 (C), 119.2 (C), 123.9 (C), 124.1 (CH x 2), 129.1 (CH x 2), 137.7 (C), 143.3 (C), 146.1 (C), 150.5 (C), 157.7 (C), 174.5 (C), 179.5 (C). EIMS m/z (%): 571 ($[M]^+$, 100), 569 (98), 430 (44), 428 (42), 348 (41), 346 (39), 83 (42), 55 (90). HREIMS: 571.2165 (calcd for $C_{31}H_{40}O_4N^{81}Br$, $[M]^+$ 571.2120) and 569.2117 (calcd for $C_{31}H_{40}O_4N^{79}Br$, $[M]^+$ 569.2141).

3-(benzo[d][1,3]dioxol-5-yl)-2-(cyclohexylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5d)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.47mg of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 32.2 mg (59%) of **5d** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.39

(m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.02 (m, 2H), 2.46 (t, *J*= 7.6 Hz, 2H), 3.69 (m, 1H), 4.57 (d, *J*= 8.4 Hz, 1H), 6.00 (bs, 1H), 6.86 (bs, 2H), 6.91 (bs, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 34.0 (CH_2), 52.5 (CH), 97.2 (C), 101.3 (CH_2), 108.7 (CH), 109.6 (CH), 119.2 (C), 122.2 (CH x 2), 123.7 (C), 124.6 (C), 142.3 (C), 147.0 (C), 148.0 (C), 150.1 (C), 157.5 (C), 174.0 (C), 179.8 (C). EIMS m/z (%): 535 ($[M]^+$, 88), 534 (100), 452 (70), 395 (46), 312 (59), 256 (32), 172 (27), 121 (45), 83 (60). HREIMS: 535.2922 (calcd for $C_{32}H_{41}O_6N$, $[M]^+$ 535.2934).

2-(cyclohexylamino)-3-(3-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5e)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 11.05 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 33.1 mg (64%) of **5e** as a dark blue oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.38 (m, 3H), 1.48 (m, 2H), 1.64 (m, 1H), 1.74 (m, 2H), 2.03 (m, 2H), 2.46 (t, *J*= 7.7 Hz, 2H), 3.71 (m, 1H), 4.62 (d, *J*= 7.9 Hz, 1H), 6.86 (bs, 1H), 7.17(m, 1H), 7.21 (d, *J*= 7.9 Hz, 1H), 7.31 (m, 1H), 7.39 (m, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 34.0 (CH_2), 52.5 (CH), 96.0 (C), 114.3 (CH, J_{CF}= 21.0 Hz), 115.8 (CH, J_{CF}= 22.1 Hz), 119.1 (C), 121.2 (C), 124.3 (CH, J_{CF}= 2.8 Hz), 130.3 (CH, J_{CF}= 8.5 Hz), 132.4 (C, J_{CF}= 8.5 Hz), 142.8 (C), 150.3 (C), 157.4 (C), 163.0 (C, J_{CF}= 241.3 Hz), 174.3 (C), 179.6 (C). EIMS m/z (%): 509 ($[M]^+$, 100), 427 (44), 369

(62), 286 (58), 83 (21), 55 (59). HREIMS: 509.2910 (calcd for $C_{31}H_{40}O_4NF$, $[M]^+$ 509.2941).

3-(4-chlorophenyl)-2-(cyclohexylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5f)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 14.78 mg of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 31.2 mg (57%) of **5f** as a dark blue oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 7.1 Hz, 3H), 1.24 (bs, 18H), 1.42 (m, 3H), 1.63 (m, 2H), 1.73 (m, 2H), 2.02 (m, 2H), 2.45 (t, J= 7.8 Hz, 2H), 4.50 (d, *J*= 8.4 Hz, 1H), 6.79 (bs, 1H), 7.44 (dd, J= 8.7, 1.9 Hz, 2H), 8.32 (dd, *J*= 8.7, 1.9 Hz, 2H). ^{13}C-NMR (250 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.3 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 33.9 (CH_2), 52.5 (CH), 96.1 (C), 119.2 (C), 124.4 (C), 126.9 (C), 128.6 (CH x 2), 130.1 (CH x 2), 140.1 (C), 142.7 (C), 150.2 (C), 157.4 (C), 174.3 (C), 179.7 (C). EIMS m/z (%): 526 ($[M]^+$, 100), 294 (96), 265 (30), 153 (95), 126 (55), 110 (25), 83 (87), 55 (73). HREIMS: 526.2654 (calcd for $C_{31}H_{40}O_4NCl$, $[M]^+$ 526.2646).

2-(cyclohexylamino)-3-(4-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5g)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 11.16 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 34.8 mg (66%) of **5g** as a dark blue

oil.[1]H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.36 (m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.02 (m, 2H), 2.46 (t, *J*= 7.4 Hz, 2H), 3.69 (m, 1H), 4.50 (bs, 1H), 6.81 (bs, 1H), 7.12 (t, *J*= 8.7 Hz, 2H), 7.40 (dd, *J*= 8.7, 5.3 Hz, 2H).[13]C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.5 (CH_2), 29.4 (CH_2), 29.5 (CH_2 x 2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 32.0 (CH_2), 34.0 (CH_2), 52.5 (CH), 96.4 (C), 115.7 (CH x 2, J_{CF}= 21.6 Hz), 119.2 (C), 124.5(C), 126.1 (C), 127.4 (C), 130.6 (CH x 2, J_{CF}= 8.1 Hz), 142.6 (C), 150.2 (C), 157.4 (C), 161.2 (C, J_{CF}= 246.3 Hz), 174.2 (C), 179.8 (C). EIMS m/z (%): 509 ($[M]^+$, 100), 426 (45), 368 (56), 286 (55), 83 (31), 55 (64). HREIMS: 509.2910 (calcd for $C_{31}H_{40}O_4NF$, $[M]^+$ 509.2941).

2-(cyclohexylamino)-3-(3,4-dimethoxyphenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5h)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.47mg of aldehyde (0.102 mmol), 13.0 μL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 32.2 mg (59%) of **5h** as a dark green oil.[1]H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.39 (m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.03 (m, 2H), 2.46 (t, *J*= 7.7 Hz, 2H), 3.70 (m, 1H), 3.91 (s, 6H), 4.64 (d, *J*= 8.5 Hz, 1H), 6.86 (bs, 1H), 6.92 (bd, *J*=8.3 Hz, 1H), 6.95 (dd, *J*= 8.2, 1.9 Hz, 1H), 7.04 (d, *J*= 1.8 Hz, 1H). [13]C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 34.0 (CH_2), 52.4 (CH), 56.0 (CH_3 x 2), 97.5 (C), 111.4 (CH), 112.7 (CH), 119.1 (CH), 120.7 (CH), 122.6 (C), 124.6 (C), 130.2 (C), 142.4 (C), 148.4 (C), 149.1 (C), 150.2 (C), 157.5 (C), 174.0 (C), 179.8 (C).

EIMS m/z (%): 551 ([M]$^+$, 100), 469 (11), 411 (16), 328 (11), 294 (15), 164 (25), 153 (26), 83 (10), 55 (25). HREIMS: 551.3206 (calcd for $C_{33}H_{45}O_6N$, [M]$^+$ 551.3247).

2-(cyclohexylamino)-5-hydroxy-3-(4-methoxyphenyl)-6-undecylbenzofuran-4,7-dione (5i)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 12.72 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.7 mg (56%) of **5i** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.25 (bs, 18H), 1.36 (m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.72 (m, 2H), 2.02 (m, 2H), 2.46 (t, *J*= 7.6 Hz, 2H), 3.69 (m, 1H), 3.84 (s, 3H), 4.55 (d, *J*= 8.4 Hz, 1H), 6.83 (bs, 1H), 6.97 (d, *J*= 8.8 Hz, 2H), 7.36 (d, *J*= 8.8 Hz, 2H).^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.2 (CH_2), 34.0 (CH_2), 52.5 (CH), 55.3 (CH_3), 97.4 (C), 114.3 (CH x 2), 119.2 (C), 122.2 (C), 124.7 (C), 130.1(CH x 2), 142.4 (C), 150.1 (C), 157.4 (C), 158.9 (C), 174.0 (C), 179.8 (C). EIMS *m/z* (%): 521 ([M]$^+$, 100), 381 (15), 299 (13), 294 (35), 153 (46), 135 (37), 83 (13), 56 (25), 55 (28). HREIMS: 521.3154 (calcd for $C_{32}H_{43}O_5N$, [M]$^+$ 521.3141).

2-(cyclohexylamino)-5-hydroxy-3-(thiophen-3-yl)-6-undecylbenzofuran-4,7-dione (5j)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 9.10 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-

TLC with 20% hexanes/EtOAc to yield 28.9 mg (57%) of **5j** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.39 (m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.03 (m, 2H), 2.46 (t, *J*= 7.7 Hz, 2H), 3.70 (m, 1H), 3.91 (s, 6H), 4.64 (d, *J*= 8.5 Hz, 1H), 6.86 (bs, 1H), 6.92 (bd, 1H), 6.95 (d, *J*= 1.9 Hz, 1H), 7.04 (d, *J*= 1.8 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 3), 31.9 (CH_2), 52.5 (CH), 93.2 (C), 119.2 (C), 122.5 (CH), 125.5 (CH), 128.7 (C), 130.2 (C), 139.5 (CH), 142.3 (C), 150.2 (C), 157.3 (C), 174.1 (C), 179.8 (C). EIMS *m/z* (%): 497 ($[M]^+$, 38), 448 (21), 300 (31), 294 (61), 285 (76), 253 (29), 239 (53), 197 (32), 155 (42), 153 (92), 129 (40), 97 (33), 83 (44), 72 (60), 55 (100). HREIMS: 497.2577 (calcd for $C_{29}H_{39}O_4NS$, $[M]^+$ 497.2600).

2-(cyclohexylamino)-5-hydroxy-3-(pyridine-3-yl)-6-undecylbenzofuran-4,7-dione (5k)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 10.00 μL of aldehyde (0.102 mmol), 13.0 μL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 4.3 mg (9%) of **5k** as a dark greenoil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.38 (m, 3H), 1.50 (m, 2H), 1.64 (m, 1H), 1.74 (m, 2H), 2.04 (m, 2H), 2.48 (t, *J*= 7.6 Hz, 2H), 3.71 (m, 1H), 4.71 (d, *J*= 8.0 Hz, 1H), 7.36 (m, 1H), 7.81 (d, *J*= 7.6 Hz, 1H), 8.51 (bs, 1H), 8.76 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.3 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 4), 31.9 (CH_2), 33.9 (CH_2), 52.6 (CH), 93.2 (C), 119.4 (C), 123.6 (C), 124.4 (CH), 127.0 (C), 136.6 (CH), 143.2 (C), 147.7 (CH), 149.2 (CH), 150.9 (C), 157.5 (C), 174.5

(C), 179.8 (C). EIMS m/z (%): 492 ([M]$^+$, 100), 269 (16), 268 (11), 163 (6), 91 (5), 83 (9), 67 (23), 55 (25). HREIMS: 492.2990 (calcd for $C_{30}H_{40}O_4N_2$, [M]$^+$ 492.2988).

2-(cyclohexylamino)-3-(furan-3-yl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (5l)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 8.78 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 19.6 mg (40%) of **5l** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.25 (bs, 18H), 1.39 (m, 3H), 1.49 (m, 2H), 1.63 (m, 1H), 1.73 (m, 2H), 2.06 (m, 2H), 2.46 (t, *J*= 7.6 Hz, 2H), 3.70 (m, 1H), 4.41 (d, *J*= 8.1 Hz, 1H), 6.71 (d, *J*= 0.9 Hz, 1H), 7.52 (bs, 1H), 7.82 (bs, 1H).^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.7 (CH_2), 25.4 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2x 3), 31.9 (CH_2), 34.0 (CH_2), 52.6 (CH), 89.5 (C), 110.0 (CH), 114.8 (C), 119.3 (C), 124.4 (C), 140.6 (CH), 142.7 (C), 143.3 (CH), 150.2 (C), 157.3 (C), 174.1 (C), 179.7 (C). EIMS m/z (%): 481 ([M]$^+$, 53), 415 (14), 259 (9), 153 (10), 99 (16), 83 (17), 67 (28), 56 (100). HREIMS: 481.2810 (calcd for $C_{29}H_{39}O_5N$, [M]$^+$ 481.2828).

2'-(cyclohexylamino)-5'-hydroxy-6'-undecyl-[2,3'-bibenzofuran]-4',7'-dione (5m)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 12.36 µL of aldehyde (0.102 mmol), 13.0 µL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 34.5 mg (64%) of **5m** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J*= 6.8 Hz, 3H), 1.25 (bs, 18H), 1.47 (m, 5H), 1.66 (m, 1H), 1.79 (m, 2H), 2.11 (m, 2H), 2.47 (t, *J*= 7.6 Hz, 2H), 3.87 (m,

1H), 6.29 (d, *J*= 8.2 Hz, 1H), 6.95 (bs, 1H), 7.23 (m, 2H), 7.41 (m, 1H), 7.56 (m, 1H), 7.61 (s, 1H).^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 24.6 (CH_2), 25.4 (CH_2), 28.5 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.6 (CH_2), 29.7 (CH_2 x 3), 31.9 (CH_2), 33.7 (CH_2), 52.2 (CH), 88.0 (C), 103.4 (CH), 110.2 (CH), 119.3 (C), 120.8 (CH), 122.5 (C), 123.4 (CH), 123.7 (CH), 129.0 (C), 142.3 (C), 149.0 (C), 150.1 (C), 153.5 (C), 158.0 (C), 173.8 (C), 179.1 (C). EIMS m/z (%): 531 ($[M]^+$, 100), 449 (71), 391 (49), 391 (49), 308 (77), 252 (15), 118 (15), 83 (16), 55 (52).HREIMS: 531.2979 (calcd for $C_{33}H_{41}O_5N$, $[M]^+$ 531.2985).

2-(cyclohexylamino)-5-hydroxy-3-propyl-6-undecylbenzofuran-4,7-dione (5n)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 9.20 μL of aldehyde (0.102 mmol), 13.0 μL of cyclohexyl isocyanide (0.102 mmol) and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 23.7 mg (51%) of **5n** as a dark blue oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.2 Hz, 3H), 0.91 (t, *J*= 7.6 Hz, 3H), 1.25 (bs, 18H), 1.39 (m, 6H), 1.47 (m, 2H), 1.66 (m, 1H), 1.77 (m, 2H), 2.06 (m, 2H), 2.46 (m, 4H), 6.85 (bs, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_3), 14.3 (CH_3), 22.6 (CH_2), 22.7 (CH_2 x 2), 24.7 (CH_2), 24.7 (CH_2), 25.6 (CH_2), 27.8 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 3), 29.7 (CH_2 x 2), 31.9 (CH_2), 34.2 (CH_2), 52.9 (CH), 98.5 (C), 119.5 (C), 126.3 (C), 142.3 (C), 149.8 (C), 157.8 (C), 173.8 (C), 180.5 (C). EIMS m/z (%): 457 ($[M]^+$, 100), 374 (23), 359 (35), 317 (35), 233 (36), 56 (65). HREIMS: 457.3185 (calcd for $C_{28}H_{43}O_4N$, $[M]^+$ 457.3192).

2-(cyclohexylamino)-3-hexyl-5-hydroxy-6-undecylbenzofuran-4,7-dione (5o)

Following the general procedure described above, 30 mg of embelin (0.102 mmol), 15.00 μL of aldehyde (0.102 mmol), 13.0 μL of cyclohexyl isocyanide (0.102 mmol)

and 1.8 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 20.7 mg (40%) of **5o** as a dark blue oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (m, 6H), 1.25 (bs, 18H), 1.29 (bs, 10H), 1.38 (m, 3H), 1.49 (m, 2H), 1.63 (m, 2H), 1.75 (m, 2H), 2.02 (m, 1H), 2.44 (m, 2H), 3.60 (bs, 1H). 3.86 (bs, 1H), 6.77 (bs, 1H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3 x 2), 22.6 (CH_2), 22.7 (CH_2 x 3), 24.8 (CH_2), 25.5 (CH_2), 28.6 (CH_2), 29.0 (CH_2), 29.3 (CH_2), 29.4 (CH_2 x 2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 3), 31.6 (CH_2), 31.9 (CH_2), 34.2 (CH_2), 52.9 (CH), 98.9 (C), 119.5 (C), 126.2(C), 142.3 (C), 149.8 (C), 157.7 (C), 173.8 (C), 180.5 (C). EIMS m/z (%): 457 ($[M]^+$, 100), 374 (23), 359 (35), 317 (35), 233 (36), 56 (65). HREIMS: 499.3661 (calcd for $C_{31}H_{49}O_4N$, $[M]^+$ 499.3662).

2-(benzylamino)-5-hydroxy-3-phenyl-6-undecylbenzofuran-4,7-dione (6a)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 6.9 μL of benzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.2 mg (86%) of **6a** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.50 (m, 2H), 2.47 (t, *J*= 7.7 Hz, 2H), 4.57 (d, *J*= 6.0 Hz, 2H), 5.04 (t, *J*= 6.0 Hz, 1H), 7.33 (m, 6H), 7.41 (m, 4H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.6 (CH_2), 97.6 (C), 119.1 (C), 127.6 (CH), 127.7 (C), 127.8 (CH x 2), 128.0 (CH), 128.7 (CH x 2), 128.8 (CH x 2), 128.9 (CH x 2), 129.8 (C), 137.6 (C), 142.9 (C), 150.4 (C), 157.3 (C), 174.4 (C), 179.6 (C); EIMS m/z (%) 499 (M^+, 55), 351 (21), 153 (40), 90 (100); HREIMS 499.2722 (calcd. for $C_{32}H_{37}O_4N$ (M^+) 499.2723).

2-(benzylamino)-5-hydroxy-3-(4-nitrophenyl)-6-undecylbenzofuran-4,7-dione (6b)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 10.3 mg of 4-nitrobenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with DCM to yield 19.4 mg (52%) of **6b** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.48 (m, 2H), 2.47 (t, *J*= 7.7 Hz, 2H), 4.61 (d, *J*= 5.8 Hz, 2H), 5.16 (t, *J*= 5.8 Hz, 1H), 6.87 (bs, 1H), 7.34 (m, 5H), 7.61 (d, *J*= 8.6 Hz, 1H), 8.24 (d, *J*= 8.6 Hz, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 95.3 (C), 119.2 (C), 123.8 (C), 124.1 (CH x 2), 127.8 (CH x 2), 128.3 (CH), 129.0 (CH x 2), 129.1 (CH x 2), 137.0 (C), 137.2 (C), 143.6 (C), 146.3 (C), 150.6 (C), 157.5 (C), 174.7 (C), 179.4 (C); EIMS m/z (%) 544 (M^+, 55), 403 (24), 294 (13); HREIMS 544.2574 (calcd. for $C_{32}H_{36}O_6N_2$ (M^+) 544.2573).

2-(benzylamino)-3-(4-bromophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6c)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 12.3 mg of 4-bromobenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.7 mg (76%) of **6c** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.50 (m, 2H), 2.46 (t, *J*= 7.6 Hz, 2H), 4.56 (d, *J*= 5.8 Hz, 2H), 4.98 (bs, 1H), 7.30 (m, 7H), 7.52 (d, *J*= 8.2Hz, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7

(CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 96.4 (C), 119.2 (C), 121.4 (C), 124.3 (C), 127.8 (CH x 2), 128.1 (CH), 128.8 (C), 128.9 (CH x 2), 130.4 (CH x 2), 132.0 (CH x 2), 137.4 (C), 143.0 (C), 150.4 (C), 157.2 (C), 174.4 (C), 179.5 (C); EIMS m/z (%) 578 (M^+, 97), 438 (32), 278 (10); HREIMS 579.1819 (calcd. for $C_{32}H_{36}O_4N^{81}Br$ (M^+) 579.1807), 577.1823 (calcd. for $C_{32}H_{36}O_4N^{79}Br$ (M^+) 577.1828).

3-(benzo[d][1,3]dioxol-5-yl)-2-(benzylamino)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6d)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 10.2mg of piperonal (0.068 mmol), 8.3 µL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 33.8 mg (92%) of **6d** as a dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.25 (bs, 16H), 1.48 (m, 2H), 2.45 (t, *J*= 7.8 Hz, 2H), 4.54 (d, *J*= 5.9 Hz, 2H), 5.00 (bs, 1H), 5.96 (s, 2H), 6.81 (d, *J*= 8.1 Hz, 1H), 6.84 (dd, *J*= 1.2, 7.9 Hz, 1H), 6.89 (d, *J*= 1.30 Hz, 1H), 7.31 (m, 5H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.6 (CH_2), 97.6 (C), 101.3 (CH_2), 108.6 (CH), 109.5 (CH), 119.2 (C), 122.2 (CH), 123.3 (C), 124.5 (C), 127.8 (CH x 2), 128.0 (CH), 128.9 (CH x 2), 137.6 (C), 142.6 (C), 147.1 (C), 148.0 (C), 150.3 (C), 157.3 (C), 174.3 (C), 179.6 (C); EIMS m/z (%) 543 (M^+, 87), 402 (30), 294 (25), 91 (100); HREIMS 543.2606 (calcd. for $C_{33}H_{37}O_6N$ (M^+) 543.2621).

2-(benzylamino)-3-(3-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6e)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 7.2 µL of 4-fluorobenzaldehyde (0.068 mmol), 8.3 µL of *t*-butyl isocyanide (0.068 mmol)

and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 28.3 mg (80%) of **6e** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.9 Hz, 3H), 1.26 (bs, 16H), 1.49 (m, 2H), 2.47 (t, *J*= 7.7 Hz, 2H), 4.58 (d, *J*= 5.9 Hz, 2H), 5.04 (bs, 1H), 6.87 (bs, 1H), 7.16 (dt, *J*= 2.2, 9.9 Hz, 1H), 7.19 (d, *J*= 7.8 Hz, 1H), 7.33 (m, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.6 (CH_2), 96.3 (C), 114.5 (CH, J_{C-F}= 22.8 Hz), 115.8 (CH, J_{C-F}= 22.3 Hz), 119.1 (C), 124.3 (CH, J_{C-F}= 2.5 Hz), 127.7 (CH x 2), 128.1 (CH), 128.7 (C), 128.9 (CH x 2), 130.3 (CH, J_{C-F}= 8.9 Hz), 131.9 (C), 137.4 (C), 143.0 (C), 150.4 (C), 157.3 (C), 162.9 (C, J_{C-F}= 246.0 Hz), 174.5 (C), 179.4 (C); EIMS m/z (%) 517 (M^+, 69), 377 (29), 91 (100); HREIMS 517.2637 (calcd. for $C_{32}H_{36}O_4NF$ (M^+) 517.2628).

2-(benzylamino)-3-(4-chlorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6f)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 9.6 mg of 4-chlorobenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.4 mg (81%) of **6f** as an amorphous dark blue solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.26 (bs, 16H), 1.49 (m, 2H), 2.46 (t, *J*= 7.9 Hz, 2H), 4.56 (d, *J*= 5.9 Hz, 2H), 4.98 (t, *J*=6.0 Hz, 1H), 7.30 (m, 3H), 7.35 (m, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.5 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 96.4 (C), 119.2 (C), 127.7 (CH x 2), 128.1 (CH), 128.3 (C), 128.9 (CH x 2), 129.0 (CH x 2), 130.1 (CH x 2), 132.7 (C), 133.3 (C), 137.4

(C), 142.9 (C), 150.4 (C), 157.2 (C), 174.4 (C), 179.5 (C); EIMS m/z (%) 532 (M^+, 99), 392 (41), 90 (100); HREIMS 535.2314 (calcd. for $C_{32}H_{36}O_4N^{37}Cl$ (M^+) 535.2303), 533.2360 (calcd. for $C_{32}H_{36}O_4N^{35}Cl$ (M^+) 533.2333).

2-(benzylamino)-3-(4-fluorophenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6g)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 7.3 μL of 4-fluorobenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 10% hexanes/EtOAc to yield 27.3 mg (76%) of **6g** as a dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.25 (bs, 16H), 1.49 (m, 2H), 2.46 (t, *J*= 7.8 Hz, 2H), 4.55 (d, *J*= 6.2 Hz, 2H), 4.94 (t, *J*= 5.9 Hz, 1H), 6.84 (bs, 1H), 7.09 (t, *J*= 8.8 Hz, 2H), 7.30 (m, 3H), 7.37 (m, 4H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 96.7 (C), 115.9 (CH x 2, $J_{C\text{-}F}$= 22.1 Hz), 119.2 (C), 124.5 (C), 125.7 (C, $J_{C\text{-}F}$= 3.1 Hz), 127.7 (CH x 2), 128.0 (CH), 128.9 (CH x 2), 130.7 (CH x 2, $J_{C\text{-}F}$= 8.2 Hz), 137.5 (C), 142.9 (C), 150.4 (C), 157.2 (C), 162.0 (C, $J_{C\text{-}F}$= 248.8 Hz), 174.4 (C), 179.6 (C); EIMS m/z (%) 517 (M^+, 100), 376 (35), 90 (94); HREIMS 517.2619 (calcd. for $C_{32}H_{36}O_4NF$ (M^+) 517.2628).

2-(benzylamino)-3-(3,4-dimethoxyphenyl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6h)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 11.3 mg of 3,4-dimethoxybenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 25.0 mg (66%) of **6h** as a dark

green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.0 Hz, 3H), 1.26 (bs, 16H), 1.50 (m, 2H), 2.47 (t, *J*= 7.8 Hz, 2H), 3.86 (s, 3H), 3.89 (s, 3H), 4.58 (d, *J*= 6.0 Hz, 2H), 4.96 (bs, 1H), 6.88 (d, *J*= 8.3 Hz, 1H), 6.94 (dd, *J*= 2.0, 8.2 Hz, 1H), 6.99 (d, *J*= 1.90 Hz, 1H), 7.32 (m, 5H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.8 (CH_2), 55.9 (CH_3), 56.0 (CH_3), 98.0 (C), 111.3 (CH), 112.6 (CH), 119.1 (C), 120.8 (CH), 122.2 (C), 124.5 (C), 127.7 (CH x 2), 128.0 (CH), 128.9 (CH x 2), 137.7 (C), 142.7 (C), 148.6 (C), 149.1 (C), 150.3 (C), 157.3 (C), 174.3 (C), 179.7 (C); EIMS m/z (%) 559 (M^+, 36), 430 (19), 294 (34), 91 (100); HREIMS 559.2948 (calcd. for $C_{34}H_{41}O_6N$ (M^+) 559.2934).

2-(benzylamino)-5-hydroxy-3-(4-methoxyphenyl)-6-undecylbenzofuran-4,7-dione (6i)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 8.3 μL of 4-methoxybenzaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 31.4 mg (87%) of **6i** as dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.49 (m, 2H), 2.46 (t, *J*= 7.7 Hz, 2H), 3.81 (s, 3H), 4.56 (d, *J*= 5.6 Hz, 2H), 4.95 (bs, 1H), 6.84 (bs, 1H), 6.94 (d, *J*= 8.8 Hz, 1H), 7.32 (m, 7H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 55.3 (CH_3), 97.7 (C), 114.3 (CH x 2), 119.2 (C), 121.8 (C), 127.8 (CH x 2), 127.9 (CH), 128.9 (CH x 2), 130.1 (CH x 2), 134.0 (C), 137.6 (C), 142.6 (C), 150.3 (C), 157.3 (C), 159.0 (C), 174.3 (C), 179.7 (C); EIMS m/z (%) 529

(M^+, 46), 528 (95), 439 (25), 388 (28), 90 (100); HREIMS 529.2847 (calcd. for $C_{33}H_{39}O_5N$ (M^+) 529.2828).

2-(benzylamino)-5-hydroxy-3-(thiophen-3-yl)-6-undecylbenzofuran-4,7-dione (6j)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 6.0 μL of 3-thiophenecarboxaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 29.2 mg (88%) of **6j** as dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.49 (m, 2H), 2.47 (t, *J*= 7.7 Hz, 2H), 4.60 (d, *J*= 5.9 Hz, 2H), 5.03 (bs, 1H), 6.90 (bs, 1H), 7.34 (m, 7H), 7.43 (dd, *J*= 1.2, 2.9 Hz, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 93.5 (C), 119.2 (C), 122.8 (CH), 124.3 (C), 126.1 (CH), 127.8 (CH x 3), 128.0 (CH), 128.9 (CH x 2), 129.8 (C), 137.5 (C), 142.6 (C), 150.4 (C), 157.5 (C), 174.3 (C), 179.6 (C); EIMS m/z (%) 505 (M^+, 85), 389 (31), 294 (45), 90 (100); HREIMS 505.2305 (calcd. for $C_{30}H_{35}O_4S$ (M^+) 505.2287).

2-(benzylamino)-5-hydroxy-3-(pyridin-3-yl)-6-undecylbenzofuran-4,7-dione (6k)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 6.4 μL of 3-pyridinecarboxaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with DCM to yield 17.2 mg (51%) of **6k** as dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.48 (m, 2H), 2.48 (t, *J*= 7.6 Hz, 2H), 4.59 (d, *J*= 5.6 Hz, 2H), 5.28 (bs, 1H), 7.32 (m, 6H), 7.79 (d, *J*= 6.6 Hz, 1H), 8.47 (s, 1H), 8.74 (s, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2),

22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.7 (CH_2), 95.3 (C), 119.4 (C), 127.7 (C), 127.9 (CH x 4), 128.1 (CH x 2), 128.8 (C), 129.9 (CH x 3), 137.3 (C), 143.4 (C), 151.1 (C), 157.5 (C), 174.6 (C), 179.7 (C); EIMS m/z (%) 500 (M^+, 94), 409 (16), 360 (40), 91 (100); HREIMS 500.2660 (calcd. for $C_{31}H_{36}O_4N_2$ (M^+) 500.2675).

2-(benzylamino)-3-(furan-3-yl)-5-hydroxy-6-undecylbenzofuran-4,7-dione (6l)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 5.9 µL of 3-furancarboxaldehyde (0.068 mmol), 8.3 µL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 12.7 mg (38%) of **6l** as a dark green oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J*= 6.1 Hz, 3H), 1.26 (bs, 16H), 1.49 (m, 2H), 2.47 (t, *J*= 7.4 Hz, 2H), 4.61 (d, *J*= 5.7 Hz, 2H), 6.69 (s, 1H), 6.89 (s, 1H), 7.35 (m, 5H), 7.49 (d, *J*= 1.3, 1H), 7.81 (d, *J*= 1.0 Hz, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.9 (CH_2), 89.8 (C), 109.9 (CH), 114.4 (C), 119.3 (C), 124.5 (C), 127.7 (CH x 2), 128.1 (CH), 128.9 (CH x 2), 137.5 (C), 140.7 (CH), 142.9 (C), 143.3 (C), 150.3 (C), 157.2 (C), 174.3 (C), 179.6 (C); EIMS m/z (%) 489 (M^+, 100), 398 (11), 418 (16), 350 (11), 349 (23); HREIMS 489.2527 (calcd. for $C_{30}H_{35}O_5N$ (M^+) 489.2515).

2'-(benzylamino)-5'-hydroxy-6'-undecyl-[2,3'-bibenzofuran]-4',7'-dione (6m)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 8.2 µL of 2-Benzofurancarboxaldehyde (0.068 mmol), 8.3 µL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by

preparative-TLC with 20% hexanes/EtOAc to yield 23.2 mg (63%) of **6m** as dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J*= 7.1 Hz, 3H), 1.26 (bs, 16H), 1.50 (m, 2H), 2.49 (t, *J*= 7.8 Hz, 2H), 4.79 (d, *J*= 6.1 Hz, 2H), 6.65 (t, *J*= 6.2 Hz, 1H), 6.97 (bs, 1H), 7.23 (m, 2H), 7.34 (m, 1H), 7.41 (m, 5H), 7.58 (m, 1H), 7.65 (s, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.5 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 47.2 (CH_2), 88.4 (C), 103.1 (CH), 110.4 (CH), 119.4 (C), 120.9 (CH), 122.5 (C), 123.4 (CH), 123.9 (CH), 127.5 (CH x 2), 128.0 (CH), 128.9 (C), 129.0 (CH x 2), 137.4 (C), 142.7 (C), 148.7 (C), 150.3 (C), 153.6 (C), 158.0 (C), 174.0 (C), 179.0 (C); EIMS m/z (%) 544 (M^+, 55), 403 (24), 294 (13); HREIMS 539.2692 (calcd. for $C_{34}H_{37}O_5N$ (M^+) 539.2672).

2-(benzylamino)-5-hydroxy-3-propyl-6-undecylbenzofuran-4,7-dione (6n)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 6.2 μL of butyraldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180^{o}C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 21.8 mg (69%) of **6n** as dark green oil.^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.88 (m, 6H), 1.25 (bs, 18H), 1.48 (m, 2H), 1.52 (m, 2H), 2.44 (m, 2H), 4.30 (t, *J*= 5.8 Hz, 1H), 4.55 (d, *J*= 6.1 Hz, 2H), 7.34 (m, 5H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 13.7 (CH_3), 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2 x 2), 24.5 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.5 (CH_2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.9 (CH_2), 48.2 (CH_2), 98.3 (C), 119.5 (C), 126.3 (C), 127.7 (CH x 2), 127.9 (CH), 128.9 (CH x 2), 138.0 (C), 142.6 (C), 150.0 (C), 157.7 (C), 174.0 (C), 180.4 (C); EIMS m/z (%) 465 (M^+, 62), 376 (14), 91 (100); HREIMS 465.2903 (calcd. for $C_{29}H_{39}O_4N$ (M^+) 465.2879).

2-(benzylamino)-3-hexyl-5-hydroxy-6-undecylbenzofuran-4,7-dione (6o)

Following the general procedure described above, 20 mg of embelin (0.068 mmol), 9.5 μL of heptanaldehyde (0.068 mmol), 8.3 μL of *t*-butyl isocyanide (0.068 mmol) and 1.2 mg of EDDA (10 mol%) in 2 mL of DCE were irradiated for 15 min at 180°C. The solvent was removed under vacuum, and the crude product was purified by preparative-TLC with 20% hexanes/EtOAc to yield 13.8 mg (40%) of **6o** as dark green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.87 (m, 6H), 1.25 (bs, 18H), 1.46 (m, 4H), 1.63 (m, 4H), 2.44 (t, *J*= 7.8 Hz, 4H), 4.55 (d, *J*= 6.1 Hz, 2H), 7.34 (m, 5H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 14.0 (CH_3), 14.1 (CH_3), 22.6 (CH_2), 22.7 (CH_2), 28.6 (CH_2), 28.9 (CH_2), 29.3 (CH_2), 29.4 (CH_2 x 2), 29.5 (CH_2 x 2), 29.6 (CH_2 x 2), 29.7 (CH_2 x 2), 31.6 (CH_2), 31.9 (CH_2), 48.2 (CH_2), 99.1 (C), 119.7 (C), 126.3 (C), 127.7 (CH x 2), 127.9 (CH), 128.9 (CH x 2), 138.5 (C), 142.9 (C), 150.2 (C), 157.4 (C), 174.1 (C), 180.2 (C); EIMS m/z (%) 507 (M^+, 100), 436 (9), 367 (16); HREIMS 507.3345 (calcd. for $C_{32}H_{45}O_4N$ (M^+) 507.3349).

4.3 Biological evaluation

4.3.1. Inhibition of human CK2 holoenzyme

All compounds in this study were tested for their inhibitory activity towards the human CK2 holoenzyme following the procedure described earlier [24]. The synthetic peptide (RRRDDDSDDD) by CK2 was used as the substrate, which is reported to be most efficiently phosphorylated by CK2. The purity of the CK2 holoenzyme was superior to 99 %. For initial testing, inhibition was determined relative to the control at inhibitor concentrations of 10 μM in DMSO as a solvent. The reaction with pure solvent without inhibitor was used as a positive control and set to 0% inhibition. Reactions without CK2 were used as negative control and were taken as 100% inhibition. IC_{50} values were determined by measuring CK2 inhibition at eight different concentrations of inhibitors ranging from 0.001 to 100 μM in appropriate intervals and calculated from the resulting

dose-response curve. The capillary electrophoresis based assay was used for testing the inhibitors of human CK2 as described earlier [29]. IC_{50} values were calculated from the resulting dose-response curves, Prism 6 (GraphPad Software, San Diego, CA, USA) was used to evaluate the IC_{50} values. For the determination of the mode of inhibition, the ATP concentration in the assay buffer was varied to 5, 10, 50 and 100 μM, while the rest of the procedure was identical to the IC_{50} determination described above.

4.3.2. Cell Culture and Proliferation

MCF7 human breast adenocarcinoma cell, provided by the Department of Clinical Radiology of the University Hospital Münster, Germany, was cultured in DMEM High glucose medium supplemented with 2 mM L-Glutamin and 10% FCS [30]. Cells were seeded at a density of 5.0×10^4 cells per well into 24-well culture plates. After overnight incubation, seeding medium was removed and replaced with fresh medium containing one of the tested compounds at 0.1, 1, 5 or 10 μM. DMSO, at a final concentration of 1%, served as a control. Cells were incubated for 24 or 48 h at 37 °C in a humidified atmosphere (5% CO_2). Cell proliferation was quantified by the EdU-click assay (Baseclick BCK-EdU555-1, Baseclick GmbH, Munich, Germany). The nucleoside analog 5-ethynyl-2′-deoxyuridine is incorporated during active DNA synthesis, and the 5-TAMRA-PEG3-azide fluorophore, used for detection, is coupled by click reaction. The results were calculated as a percent ratio of proliferating cells versus total number of untreated cells. CK2 inhibitor was assayed in triplicates, and the experiments were repeated three times.

4.3.3. Cell viability assay

The effect of CK2 inhibitors on the viability of MCF7 cells was evaluated using MTT assay [31]. This assay is a colorimetric assay, which measures the conversion of MTT into violet formazan that is produced by succinate dehydrogenase of the intact

mitochondria in viable cells. MTT assay was performed in 96-well plates. Cells were seeded at a density of 1 x 10^5 cells per well. Cells were incubated for 24 or 48 h at 37 °C in a humidified atmosphere (5% CO_2). After overnight incubation, seeding medium was removed and replaced with fresh medium containing the inhibitor at 0.1, 1, 5 or 10 μM. DMSO, at a final concentration of 1%, served as a control. Afterwards MTT reagent (Sigma Aldrich, Germany) was added at a final concentration of 0.5 mg/mL. After incubation for 2 h at 37 °C medium was discarded and 200 μL DMSO was added for solubilization the formazan. After mixing, the absorption was determined at 570 nm with a reference wavelength of 630 nm using a microplate reader. CK2 inhibitor was assayed in triplicates, and the experiments were repeated three times.

4.4. Protein Preparation and Docking.

The X-ray coordinates of human protein kinase CK2 alpha subunit in complex with the inhibitor CX-4945 (PDB 3PE1). The PDB structures were prepared for docking using the Protein Preparation Workflow (Schrodinger, LLC, New York, NY, 2018) accessible from within the Maestro program (Maestro, version 11.6; Schrodinger, LLC: New York, NY, 2018). The substrate and water molecules were removed beyond 5 Å, bond corrections were applied to the cocrystallized ligands and an exhaustive sampling of the orientations of groups was performed. Finally, the receptors were optimized in Maestro 11.6 by using OPLS3 force field before docking study. In the final stage the optimization and minimization on the ligand-protein complexes were carried out with the OPLS3 force field and the default value for rmsd of 0.30 Å for non-hydrogen atoms were used. The receptor grids were generated using the prepared proteins, with the docking grids centered on the center of the bound ligand for each receptor. A receptor grid was generated using a 1.00 van der Waals (vdW) radius scaling factor and 0.25 partial charge cutoff. The binding sites were enclosed in a grid box of 20 $Å^3$ with

default parameters and without constrains. The three-dimensional structures of the ligands to be docked were generated and prepared using LigPrep as implemented in Maestro 11.6 (LigPrep, Schrodinger, LLC: New York, NY, 2018) to generate the most probable ionization states at pH 7 ± 1 (retain original ionization state). These conformations were used as the initial input structures for the docking. In this stage a series of treatments are applied to the structures. Finally, the geometries are optimized using OPLS3 force field. These conformations were used as the initial input structures for the docking. The ligands were docked using the extra precision mode (XP) [32] without using any constraints and a 0.80 van der Waals (vdW) radius scaling factor and 0.15 partial charge cutoff. The dockings were carried out with flexibility of the residues of the pocket near to the ligand. The generated ligand poses were evaluated with empirical scoring function, GlideScore a modified version of ChemScore [33], GlideScore implemented in Glide, was used to estimate binding affinity and rank ligands [34]. The XP Pose Rank was used to select the best-docked pose for each ligand. The best correlation with the human protein kinase CK2 alpha subunit was achieved when the PDB 3PE1 was used.

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