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Original article

Synthesis and antiproliferative activity of benzofuran-based analogs of cercosporamide against non-small cell lung cancer cell lines

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ABSTRACT

A novel series of 3-methyl-1-benzofuran derivatives were synthesized and screened *in vitro* for their antiproliferative activity against two human NSCLC cell lines (NSCLC-N6 mutant p53 and A549 wild type p53). Most promising compounds presented a structural analogy with the west part of cercosporamide, a natural product of biological interest. In particular, compounds **10**, **12** and **31** showed cytotoxic activities at micromolar concentrations ($IC_{50} < 9.3$ μM) and compounds **13**, **18** and **32** displayed moderate IC_{50} values (25–40 μM).

1. Introduction

Lung cancer remains the leading cause of cancer-related deaths among both men and women worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all cases of lung cancer [1]. Antiproliferative agents-based chemotherapy (eg vinorelbine, paclitaxel, carboplatin) improves survival in advanced NSCLC whereas surgery remains the treatment of choice of early NSCLC [2]. The development of chemotherapeutic agents displaying an original mechanism of action leading to a high activity and a low toxicity is still needed. Among them, natural products represent a source of novel antiproliferative agents.

(−)-Cercosporamide (Fig. 1) is an antifungal natural agent isolated from the phytopathogen fungus *Cercosporidium henningsii* [3]. Initially this phytotoxin was found to inhibit selectively CaPkc1 kinase [4]. Recent studies showed that cercosporamide had antitumor activity through MAPK-interacting kinase 1 (Mnk1) inhibition and suppressed both growth of lung metastases [5] and acute myeloid leukemia precursors [6]. Due to these relevant biological properties, we were interested in synthesizing cercosporamide analogs based on benzofuran scaffold. Indeed, benzofuran derivatives represent an important source of antiproliferative products. Sugano et al. reported that 4-hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester derivatives showed selective and potent cytotoxicity against a tumorigenic cell line [7]. Other previous studies by Hranjec et al. have shown the antiproliferative effects of benzofuran-2-carboxamides on tumor cell lines [8]. Thus, as a part of our research program on bioactive compounds on NSCLC [9,10], we thought that benzofuran-based analogs of cercosporamide could display antiproliferative activity on two lung cancer cell lines, NSCLC-N6 and A549.

From a structural point of view, cercosporamide is a usnic acid analog that contains a dihydroxybenzamide moiety. In this way, 3-methylbenzofuran derivatives functionalized in the position 2 were synthesized while keeping a structural analogy with the west part of cercosporamide (Fig. 1). The *in vitro* antiproliferative activity of these compounds was determined by measurement of their IC_{50} values towards two cancer cell lines.

2. Results and discussion

2.1. Chemistry

Benzofuran scaffold was built from 3,5-dimethoxyphenol to achieve a suitable substitution on the benzene ring (Scheme 1).

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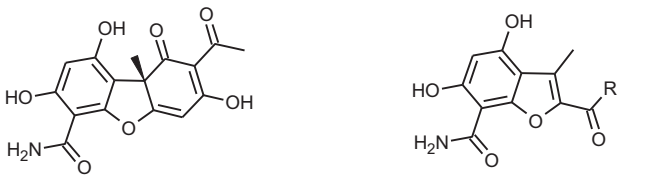


Fig. 1. Structure of (−)-cercosporamide and target compounds derived from 7-carbamoyl-4,6-dihydroxy-3-methylbenzofuran.

Phenol derivative was first acetylated by a Friedel-Crafts-type reaction using acetyl chloride and boron trichloride, at low temperature in dichloromethane [11]. Applying this reaction conditions, 2-hydroxy-4,6-dimethoxyacetophenone **1** was obtained in very good yield (97%) without isolating the *O*-acetylated by-product. Ketone **1** was then *O*-alkylated, with ethyl bromoacetate and potassium carbonate as a base in *N*,*N*-dimethylformamide (DMF) to afford ester **2** which was next cyclized with cesium carbonate in DMF under microwave radiation [12,13]. Interestingly, ethyl 4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate **3** was obtained by a one-pot reaction from ketone **1** using the previously described procedure to synthesize compound **2**. In this methodology, ester **2** was not isolated and the benzofuran derivative **3** was provided by rising the temperature from 70 °C to 100 °C. Finally, the yield was enhanced from 43% (two steps) to 84% (one step).

Bromine atom was then readily introduced at the position 7 of the benzofuran ring [14,15], by reaction of compound **3** with bromine in acetic acid at room temperature for 30 min, to afford compound **4** in excellent yield. Subsequent cyanation with copper cyanide in *N*-methylpyrrolidinone, under microwave radiation at 200 °C, gave nitrile derivative **5** in good yield [16]. Finally, hydration of nitrile function in sulfuric acid medium provided carboxamide **6**. Interestingly, as recently described in indole series [17], a direct aminocarbonylation at C-7 of benzofuran derivative **3**, using chlorosulfonyl isocyanate (CSI) as electrophilic reagent, led to compound **6** in good yield (74%) after acidic hydrolysis of the corresponding *N*-chlorosulfonylcarboxamide intermediate. In comparison, compound **6** was obtained in 71% yield using the three-step sequence from **3**: bromination–cyanation–hydration (Scheme 1).

A modification of the ethyl ester moiety of **6** into amides was performed *via* the preparation of the carboxylic acid precursor **7** and activation in the presence of coupling reagents such as 2-chloro-1-methylpyridinium iodide (CNMPI) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (Table 1).

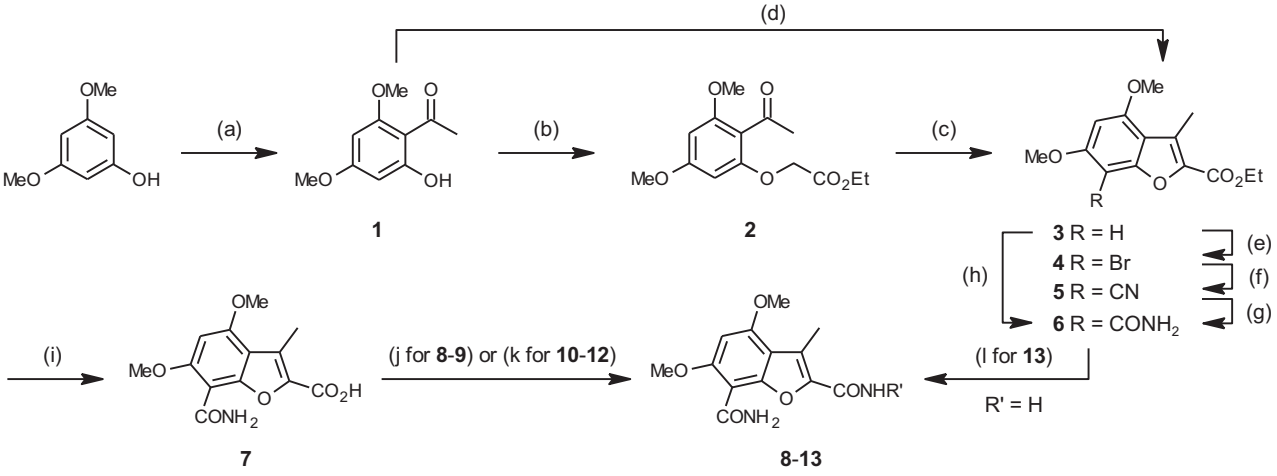
TBTU [18] was used instead of CNMPI for the synthesis of amides with less nucleophilic amines and the yields remained acceptable (55–90%).

Unsubstituted dicarboxamide derivative **13** was obtained by aminolysis of ester **6** in a methanolic solution of ammonia in 67% yield.

To prepare the aldehyde analog of ester **6**, the strategy was to carry out a reduction-oxidation sequence starting from ethyl 4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate **3** without carboxamide substitution at C-7 to avoid the reduction of the amide function in the presence of lithium aluminium hydride (LAH) in the first step (Scheme 2). It's to be noticed that the reduction of ester **4** failed due to the presence of the bromine atom on the benzofuran ring; degradation of the reaction mixture was observed.

The ethyl ester **3** was easily reduced, with LAH, to alcohol **14** which was converted to aldehyde **15** with manganese dioxide, quantitatively [7]. Unfortunately, attempt to directly access the benzofuran-7-carboxamide analog of **15**, by treatment with CSI, failed. Thus, the previously described strategy (bromination-cyanation-hydration) was applied to introduce a carboxamide function at the position 7 of benzofuran-2-carbaldehyde **15** (Scheme 2). 2-Formylbenzofuran-7-carboxamide **18** was obtained in three steps, in 17% overall yield.

The last step of the synthetic work was to prepare 4,6-dihydroxybenzofuran-7-carboxamide derivatives by demethylation of 4,6-dimethoxybenzofuran-7-carboxamide precursors. The first attempts were unsuccessful using boron tribromide [19] and compounds **6** and **8** as starting material (Scheme 3). At low temperature (−10 °C) in the presence of 2 equivalents of Lewis acid, starting material was recovered but with an excess of reagent (5–10 equiv.) and higher temperatures (room temperature to reflux), monodemethylation (UPLC-MS monitoring) and then degradation of the reaction mixture were observed. In the latter experimental



Scheme 1. Reagents and conditions: (a) AcCl, BCl_3, CH_2Cl_2, −10 °C then reflux, 3 h, 97%; (b) $BrCH_2CO_2Et$, K_2CO_3, DMF, 70 °C, 2 h, 99%; (c) Cs_2CO_3, CH_3CN, reflux, 17 h, 55%; (d) $BrCH_2CO_2Et$, K_2CO_3, DMF, 70 °C, 1 h then 100 °C, 18 h, 84%; (e) Br_2, AcOH, rt, 30 min, 93%; (f) CuCN, NMP, 200 °C, MW, 10 min, 84%; (g) H_2SO_4, 60 °C, 6 h, 91%; (h) CSI, CH_3CN, 0 °C to rt, 2 h then HCl 1M, rt, 74%; (i) NaOH, EtOH, reflux, 4.5 h, 79%; (j) CNMPI, Et_3N, CH_2Cl_2, reflux, 10 h, 64–69%; (k) TBTU, *i*-Pr_2NEt, DMF, rt, 24 h, 55–90%; (l) NH_3, MeOH, sealed tube, reflux, 60 h, 67%.

Table 1
Structures of N^2-(het)aryl-4,6-dimethoxy-3-methylbenzofuran-2,7-dicarboxamide **8**–**12**.

Compound	R′	Coupling reagent	Yield (%)[a]
8	*n*-C_3H_7	CNMPI	64
9	C_6H_5–CH_2	CNMPI	69
10	C_6H_5	TBTU	90
11	2-Pyridyl	TBTU	55
12	4-Pyridyl	TBTU	58

[a] Isolated yields.

conditions, 2-formyl-4,6-dihydroxy-3-methyl-1-benzofuran-7-carboxamide **21** was produced in low yield (21%).

To circumvent this problem, we decided to introduce the benzyl moiety as protecting group of 4,6-dihydroxybenzofuran derivatives in order to perform the cleavage in milder conditions.

In this second route, 2,4-bis(benzyloxy)-6-hydroxyacetophenone **25** was initially prepared to be cyclized into benzofuran ring as described before for the dimethoxy series (Scheme 4).

As indicated in the literature [20,21], 6-hydroxy-2,4-dibenzyloxyacetophenone **25** was furnished by an initial tribenzylation of triacetoxyphloroglucinol **22** to avoid additive *C*-benzylation by direct *O*-benzylation of phloroglucinol [22,23]. Subsequent acylation of **23**, through the formation of a mixed anhydride, afforded acetophenone **24** followed by a monodeprotection in the presence of titanium tetrachloride to give the desired compound **25** (Scheme 4).

Ethyl 4,6-dibenzyloxy-3-methyl-1-benzofuran-2-carboxylate **26** was then obtained using the method previously reported which included *O*-alkylation with ethyl bromoacetate and base-catalyzed cyclization of the alkylated product at 100 °C in DMF. In these experimental conditions, removal of ester group at the position 2 of compound **26** was observed and benzofuran by-product **27** was isolated in 20% yield after purification by column chromatography.

Interestingly, benzofuran-2-carbaldehyde **28** was prepared in good yield (83%) by the Vilsmeier–Haack formylation of **27** without C7 electrophilic attack [24]. This approach was more direct than the two-step route of reduction and oxidation from the ester **26**.

The benzyl protective groups of **26** and **28** were removed under transfer hydrogenation conditions using Pd/C and cyclohexene, in a mixture of methanol/tetrahydrofuran at reflux, to afford dihydroxybenzofuran derivatives **29** and **30** in 92% and 61% yield, respectively [25]. Efforts to install a carboxamide function at the position 7 of the analogs **29** and **30**, by the use of CSI, remained unsuccessful since a complex mixture was observed in the medium. The same reaction failed from 4,6-dibenzyloxybenzofuran-2-carbaldehyde **28**, even by rising the temperature, due to problems of solubility in acetonitrile. Finally, ester **26** reacted with CSI to provide 7-carboxamidebenzofuran derivative **31** in good yield (87%) and its benzyl groups were cleaved by catalytic hydrogenolysis to give 7-carboxamide-4,6-dihydroxybenzofuran derivative **32**.

2.2. Biological evaluation

Two cell lines were used in this study, A549 and NSCLC-N6, originating from an adenocarcinoma and an epidermoid lung cancer, respectively. The NSCLC-N6 is a cell line derived from an NSCLC of a previously untreated patient (moderately differentiated classified as T2N0M0) [26]. The A549 line was obtained from the ATCC (reference CCL-185) [27] and is known to have a wild type p53 gene, while NSCLC-N6 has a mutant p53 gene, similar to tumors *in situ*.

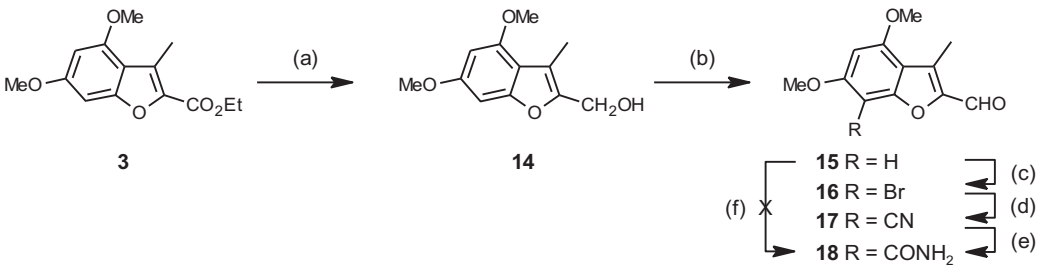
It is known that the chemoresistance observed for NSCLC is largely due to a very high proportion of cells in the G0/G1 phase of the cell cycle [28–30]. Therefore, as many anticancer drugs have a cycle-dependent activity, their effectiveness on such slowly developing tumors is limited. Consequently, we focus our research on cytostatic molecules that stop cell growth in G1-phase of the cell cycle, before initiating cells to apoptotic death. In addition, this prevents excessive toxicity *in vivo*. This approach was successfully carried out to discover the NSCLC antitumor activity of triazine **A190** [9], used as reference compound (Table 2).

Compounds **3**–**18**, **21**, **26**, **28**–**32** were tested for their *in vitro* antiproliferative activity against NSCLC-N6 and A549 human lung cancer cell lines using MTT assay. The results expressed as IC_{50} were summarized in Table 2.

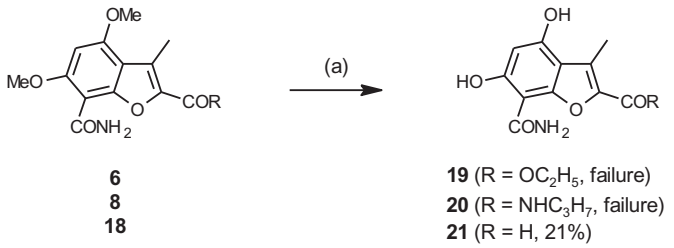
Three series (ester, aldehyde and amide), based on 3-methyl-1-benzofuran scaffold, were developed considering the substitution at the position 2 of the ring.

Among the esters, 4,6-dimethoxybenzofuran derivatives **3**–**6**, substituted or not at the position 7 were found to be inactive. Benzyl analog **26** of compound **3** remained inactive and the corresponding debenzylated derivative **29** too. Interestingly, the introduction of a carboxamide function at the position 7 of compounds **26** and **29** resulted in obtaining an antiproliferative effect of compounds **31** and **32**. Indeed, benzyl derivative **31** exhibited a cytotoxic activity against both cell lines with IC_{50} values inferior to 7.2 μM and IC_{50} values ranged from 24.0 to 32.9 μM for compound **32**.

In the second series, the same trends were observed since benzofuran-2-carboxaldehydes **15**–**17**, **28** and **30** were inactive. The only difference concerned compounds **18** and **21**. 4,6-Dimethoxybenzofuran derivatives **18** displayed IC_{50} values of 44.4 μM and 63.8 μM against NSCLC-N6 and A549, respectively, whereas ester analog **6** was totally inactive. In the contrary, 4,6-dihydroxybenzofuran derivative **21** (IC_{50} values NSCLC-N6 of



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, rt, 15 min, 95%; (b) MnO_2, CH_2Cl_2, reflux, 2 h, 98%; (c) Br_2, AcOH, rt, 2 h, 79%; (d) CuCN, NMP, 200 °C, MW, 40 min, 50%; (e) H_2SO_4, 60 °C, 6 h, 44%; (f) CSI, CH_3CN, 0 °C to rt, 15 h then HCl 1 M, rt.



Scheme 3. Reagents and conditions: (a) BBr_3, CH_2Cl_2, reflux, 10 h.

60.8 μM and IC_{50} values A549 of 108.4 μM) was less active than ester counterpart **32** (IC_{50} values NSCLC-N6 of 32.9 μM and IC_{50} values A549 of 24.0 μM).

In the last series, six 4,6-dimethoxybenzofuran-2-carboxamide derivatives **8**–**13** were tested. Among them, *N*-propylcarboxamide **8** and *N*-benzylcarboxamide **9** were inactive. *N*-(Hetero)aryl substituted carboxamides **10** (phenyl) and **12** (4-pyridyl) exhibited good cytotoxic activity (IC_{50} values inferior to 9.2 μM). Surprisingly, the design of 2-pyridyl isomer **11** of compound **12** led to a dramatic decrease in antiproliferative activity.

Finally, 2,7-dicarboxamidebenzofuran derivative **13** showed promising antitumor activity against NSCLC-N6 and A549 cell lines with IC_{50} values of 22.6 μM and 39.4 μM, respectively.

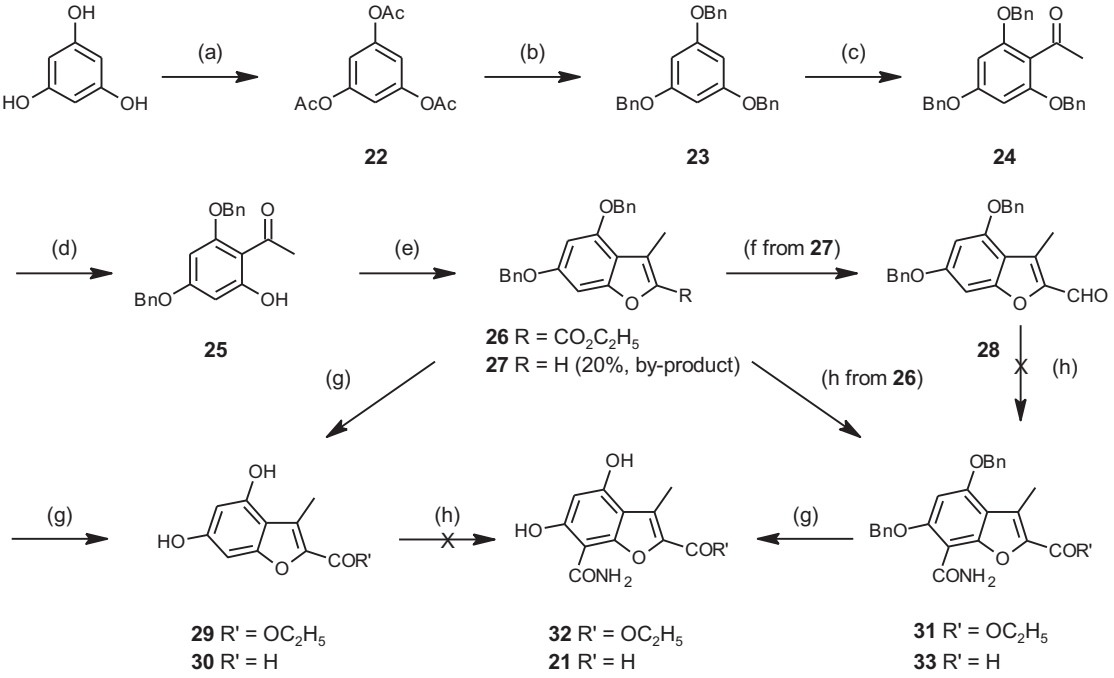
The present study investigated the biological effect of several substituents on 3-methyl-1-benzofuran ring at the position 2 (ester, aldehyde, amide), the position 7 (H, Br, CN, $CONH_2$) and the positions 4 and 6 (OMe, OBn, OH). In most cases, compounds with methoxy groups at the 4 and 6-positions of benzofuran remained inactive, except amides **10**, **12**, **13** and aldehyde **18**, more active or as active as the reference compound **A190** (Table 2). It was obvious that the antiproliferative activity was improved by the introduction of a carboxamide ($CONH_2$) group at the 7-positon (**15** *vs* **18**, **26** *vs* **31** and **29** *vs* **32**).

Structural analogy with the west part of the cercosporamide, namely 4,6-dihydroxy-3-methyl-1-benzofuran-7-carboxamide, was found on compounds **21** and **32**, displaying low antiproliferative activity against tested NSCLC cell lines, for the first one, and possessing promising activity for the second one. Generally, based on this scaffold, substitutions of the phenols by a methyl or a benzyl were preferred (**21** *vs* **18**, **32** *vs* **31**) in terms of antitumor activity. In addition, 4,6-dimethoxybenzofuran derivatives furnished also the most interesting products in dicarboxamide series (compounds **10**, **12** and **13**).

The results suggested that the design of new compounds, inspired from cercosporamide structure, was a valuable starting point to obtain *in vitro* antiproliferative agents against NSCLC cell lines.

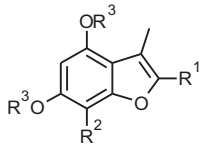
3. Conclusion

In summary, a series of 3-methyl-1-benzofuran derivatives were synthesized and screened for their antiproliferative activity against two human NSCLC cell lines (NSCLC-N6 and A549). Among them, three compounds (**10**, **12** and **31**) displayed cytotoxic activity and could be possible candidates for further evaluation. However, we retained for additional study compounds with IC_{50} values between 10 μM and 50 μM. Indeed, the goal of the project was to find cytostatic molecules *in vivo* and in this strategy a molecule whose IC_{50} value is less than 5 μM generally proves to be too toxic in animals and when its IC_{50} is greater than 60 μM, dose adjustment *in vivo* is difficult to succeed. As we proceed for reference compound **A190** (IC_{50} values NSCLC-N6 of 39.4 μM and IC_{50} values A549 of 52.6 μM), a cytostatic compound which showed a very interesting *in vivo* profile with original mechanism of action [9], compounds **13**, **18** and **32** will be selected for further pharmacological investigations. In this context, the compounds will be studied as follows: the cell responses by the observation of cell proliferation arrest to choose cytostatic agents, the induction of apoptosis by cell



Scheme 4. Reagents and conditions: (a) Ac_2O, pyridine, 120 °C, 4 h, 98%; (b) BnCl, NaH, H_2O, DMF, 0 °C to rt, 17 h, 78%; (c) AcOH, TFAA, CH_2Cl_2, 0 °C, 1.5 h, 75%; (d) $TiCl_4$, CH_2Cl_2, −5 °C, 1 h, 84%; (e) $BrCH_2CO_2Et$, K_2CO_3, DMF, 70 °C, 2 h then 100 °C, 20 h, 52%; (f) $POCl_3$, DMF, rt, 20 h then NaOH 8N, 83%; (g) Cyclohexene, Pd/C 10%, MeOH, THF, reflux, 5 h, 92% (for **29**) and 2 h, 61% (for **30** and **32**); (h) CSI, CH_3CN, 0 °C to rt, 5 h then HCl 1 M, rt, 87%.

Table 2
Inhibition effects of compounds **3-6**, **8**–**13**, **15**–**18**, **21**, **26**, **28**–**32** on the growth of NSCLC-N6 and A549 tumor cell lines *in vitro*.



Compound	R^1	R^2	R^3	NSCLC-N6[a] IC_{50} ± SEM (μM)[b]	A549[a] IC_{50} ± SEM (μM)[b]
A190[c]				39.4 ± 2.1	52.6 ± 2.8
3	$CO_2CH_2CH_3$	H	CH_3	n.a.[d]	n.a.
4	$CO_2CH_2CH_3$	Br	CH_3	>87.4	>87.4
5	$CO_2CH_2CH_3$	CN	CH_3	n.a.	n.a.
6	$CO_2CH_2CH_3$	$CONH_2$	CH_3	n.a.	n.a.
8	CONH(*n*-C_3H_7)	$CONH_2$	CH_3	>93.6	>93.6
9	$CONHCH_2C_6H_5$	$CONH_2$	CH_3	>81.4	54.8 ± 2.6
10	$CONHC_6H_5$	$CONH_2$	CH_3	<9.3	<9.3
11	CONH(2-Pyridyl)	$CONH_2$	CH_3	>84.4	>84.4
12	CONH(4-Pyridyl)	$CONH_2$	CH_3	<9.3	<9.3
13	$CONH_2$	$CONH_2$	CH_3	22.6 ± 1.3	39.4 ± 2.1
15	CHO	H	CH_3	n.a.	n.a.
16	CHO	Br	CH_3	n.a.	49.5 ± 8.0
17	CHO	CN	CH_3	n.a.	n.a.
18	CHO	$CONH_2$	CH_3	44.4 ± 4.2	63.8 ± 2.3
21	CHO	$CONH_2$	H	60.8 ± 3.4	108.4 ± 13.6
26	$CO_2CH_2CH_3$	H	Bn	>72.0	>72.0
28	CHO	H	Bn	>80.6	>80.6
29	$CO_2CH_2CH_3$	H	H	65.6 ± 6.8	68.6 ± 21.6
30	CHO	H	H	n.a.	n.a.
31	$CO_2CH_2CH_3$	$CONH_2$	Bn	<7.2	<7.2
32	$CO_2CH_2CH_3$	$CONH_2$	H	32.9 ± 1.4	24.0 ± 5.7

[a] Non-small cell lung cancer (NSCLC) cell lines.
[b] Mean from at least eight determinations. SEM: Standard Error of the Mean.
[c] **A190** the 4,6-diamino-1,2-dihydro-1-(4″-chlorophenyl)-2-(1-tricyclo[3.3.1.1^{3,4}] decyl-1,3,5-triazine hydrochloride, is used as reference compound.
[d] n.a.: not active.

cycle arrest, the molecular mechanisms involved. Finally, to confirm the *in vitro* results on the cell lines, the antitumoral effect would be also studied with an *in vivo* experiment on NSCLC-N6 xenografted nude mice. Unfortunately, no significant difference of IC_{50} values between the two cell lines (wild type p53 or mutant p53) was observed for the tested compounds. These data would indicate that p53 is not the target involved in the observed antitumor activity.

4. Experimental protocols

4.1. Chemistry

All reactions were monitored by TLC analysis using Merck silicagel 60F-254 thin-layer plates. Column chromatography was carried out on silicagel Merck 60 (70–230 mesh ASTM). Melting points were determined on an Electrothermal IA 9000 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Shimadzu IRAffinity-1 IR-FT spectrophotometer equipped with a MIRacle 10 accessory ATR. ^{1}H and ^{13}C NMR spectra were performed in DMSO-d_6 or $CDCl_3$ using a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane as internal standard and coupling constants (J) are given in Hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded using an Electrospray Ionization Method with Waters ZQ 2000 spectrometer. Microwave-promoted reactions were performed on a CEM Discover SP monomode apparatus. Elemental analyses were performed on a Thermo Scientific Elemental Analyser Flash EA 1112 and were found within ±0.4% of the theoretical values.

4.1.1. 1-(2-Hydroxy-4,6-dimethoxyphenyl)acetophenone (xanthoxylin) (**1**)

3,5-Dimethoxyphenol (15.0 g, 97.3 mmol) was dissolved in 100 mL of anhydrous dichloromethane. Boron trichloride (1 M solution in dichloromethane, 102.2 mL, 102.2 mmol) was slowly added under nitrogen at −10 °C. After 5 min, acetyl chloride (7.3 mL, 102.2 mmol) was added dropwise. The mixture was heated and kept under reflux for 3 h and was stirred overnight at room temperature. The reaction mixture was cooled at 0 °C and was slowly hydrolyzed with 1 M HCl. The organic phase was separated and the aqueous layer was extracted twice with dichloromethane. The organic layers were dried over sodium sulfate and evaporated under reduced pressure. Recrystallization from methanol afforded **1** (18.5 g, 97%) as a white powder.

R_f 0.28 (CH_2Cl_2); Mp: 80.5 °C (lit [31]. 78.5–79.5 °C); IR (KBr) ν 3102, 3021, 2945, 1612, 1595, 1572, 1422, 1366, 1269, 1219, 1207, 1155, 1111, 1082, 893, 835 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 14.04 (s, 1H, −OH), 6.07 (d, 1H, J = 2.0 Hz, H_5), 5.93 (d, 1H, J = 2.0 Hz, H_3), 3.86 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-OCH_3$), 2.62 (s, 3H, $-COCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 202.8, 166.2, 165.9, 162.8, 105.5, 93.6, 90.8, 56.0, 55.7, 32.7; MS (ESI) m/z (%): 197.1 (100) $[M + H]^+$.

4.1.2. Ethyl (2-acetyl-3,5-dimethoxyphenoxy)acetate (**2**)

To a stirred solution of compound **1** (3.95 g, 20.1 mmol) in DMF (50 mL) were successively added ethyl bromoacetate (2.46 mL, 22.2 mmol) and potassium carbonate (8.35 g, 60.4 mmol). The reaction mixture was heated to 60 °C for 20 h. After cooling, the reaction mixture was diluted with ethyl acetate and water was added. The organic layer was separated, washed twice with water and brine, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel

chromatography using dichloromethane as eluent to afford **2** (3.86 g, 68%) as a white powder.

R_f 0.13 (CH_2Cl_2); Mp: 62.0 °C; IR (KBr) ν 2974, 1732, 1694, 1452, 1416, 1287, 1231, 1200, 1135, 1020, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.15 (d, 1H, J = 2.0 Hz, H_{ar}), 5.99 (d, 1H, J = 2.0 Hz, H_{ar}), 4.62 (s, 2H, $-OCH_2-$), 4.25 (q, 2H, J = 7.2 Hz, $-C\underline{H}_2CH_3$), 3.80 (s, 6H, 2 × $-OCH_3$), 2.54 (s, 3H, $-COCH_3$), 1.29 (t, 3H, J = 7.2 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.5, 168.5, 162.3, 158.6, 156.7, 114.7, 91.9, 91.7, 66.2, 61.5, 56.0, 55.6, 32.7, 14.3; MS (ESI) m/z (%): 283.1 $[M + H]^+$. Anal. Calcd for $C_{14}H_{18}O_6$: C 59.57; H 6.43. Found: C 59.82; H 6.80%.

4.1.3. Ethyl 4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate (**3**)

4.1.3.1. Method a. Compound **2** (2.00 g, 7.1 mmol) and cesium carbonate (9.23 g, 28.3 mmol) were suspended in DMF (20 mL) in a 100 mL round bottom flask equipped with a magnetic stir bar. The opened flask was heated under microwave irradiation (30 W) at 80 °C for 90 min. After cooling, water (50 mL) and ethyl acetate (50 mL) were added. The organic phase was separated, dried over sodium sulfate and evaporated under reduced pressure. Compound **3** was obtained without further purification as a white powder (1.18 g, 63%).

4.1.3.2. Method b. To a stirred solution of compound **1** (15.50 g, 79.0 mmol) in DMF (150 mL) were added potassium carbonate (32.76 g, 237.0 mmol) and ethyl bromoacetate (9.64 mL, 86. 91 mmol). The mixture was heated at 70 °C for 1 h and then at 100 °C overnight. The reaction mixture was cooled to room temperature and filtered on Celite®. The solution was diluted with ethyl acetate (200 mL) and the organic layer was washed with brine and water, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **3** (17.64 g, 84%) as a white powder.

R_f 0.56 (CH_2Cl_2); Mp: 130.7 °C; IR (KBr) ν 3031, 2986, 2935, 1721, 1631, 1606, 1576, 1508, 1450, 1373, 1279, 1220, 1195, 1150, 1028, 807, 765 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.60 (d, 1H, J = 1.8 Hz, H_{ar}), 6.26 (d, 1H, J = 1.8 Hz, H_{ar}), 4.41 (q, 2H, J = 7.1 Hz, $-C\underline{H}_2CH_3$), 3.88 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-OCH_3$), 2.68 (s, 3H, 3$-CH_3$), 1.42 (t, 3H, J = 7.1 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.7, 160.5, 156.6, 156.5, 139.0, 127.3, 112.8, 94.6, 87.8, 60.7, 55.7, 55.5, 14.5, 11.2; MS (ESI) m/z (%): 265.0 $[M + H]^+$. Anal. Calcd for $C_{14}H_{16}O_5$: C 63.63; H 6.10. Found: C 64.01; H 6.19%.

4.1.4. Ethyl 7-bromo-4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate (**4**)

To a stirred solution of compound **3** (1.30 g, 4.92 mmol) in glacial acetic acid (50 mL) was added dropwise bromine (265 μL, 5.16 mmol) at room temperature. After 30 min, the reaction mixture was quenched with water (30 mL) and the precipitate was then filtered and washed with water. The crude product was then recrystallized in a mixture methanol/water (19:1) to afford **4** (1.57 g, 93%) as a white powder.

R_f 0.22 (CH_2Cl_2); Mp: 168–170 °C; IR (KBr) ν 2990, 1703, 1612, 1589, 1327, 1271, 1217, 1144, 1128, 978, 814, 766 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.34 (s, 1H, H_{ar}), 4.42 (q, 2H, J = 7.2 Hz, $-C\underline{H}_2CH_3$), 3.98 (s, 3H, $-OCH_3$), 3.94 (s, 3H, $-OCH_3$), 2.67 (s, 3H, $-CH_3$), 1.43 (t, 3H, J = 7.2 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.8, 168.3, 158.4, 157.2, 153.6, 120.3, 98.5, 93.0, 71.4, 61.3, 56.7, 56.2, 32.6, 14.3; MS (ESI) m/z (%): 343.0 (100) $[M + H]^+$, 345.1 $[M + H+2]^+$. Anal. Calcd for $C_{14}H_{15}BrO_5$: C 49.00; H 4.41. Found: C 49.15; H 4.43%.

4.1.5. Ethyl 7-cyano-4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate (**5**)

Compound **4** (500 mg, 1.46 mmol) and copper(I) cyanide (261 mg, 2.91 mmol) were suspended in *N*-methylpyrrolidone (5 mL) in a 10 mL glass vial equipped with a small magnetic stir bar. The vial was sealed and microwave irradiated (100 W) at 200 °C for 10 min. After cooling, the mixture was filtered and the solid was washed with methanol and dried to give a gray solid. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **5** (354 mg, 84%) as a white powder.

R_f 0.44 (CH_2Cl_2); Mp: 219.7 °C; IR (KBr) ν 2982, 2227, 1718, 1620, 1595, 1506, 1331, 1282, 1221, 1160, 1126, 1016, 976, 825, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.27 (s, 1H, H_{ar}), 4.40 (q, 2H, J = 7.1 Hz, $-C\underline{H}_2CH_3$), 4.01 (s, 3H, $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 2.65 (s, 3H, 3$-CH_3$), 1.41 (t, 3H, J = 7.1 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.4, 160.4, 159.8, 155.9, 140.4, 126.8, 113.1, 112.9, 89.8, 77.2, 61.0, 56.9, 56.0, 14.4, 11.0; MS (ESI) m/z (%): 290.0 $[M + H]^+$. Anal. Calcd for $C_{15}H_{15}NO_5$: C 62.28; H 5.23; N 4.84. Found: C 62.13; H 5.02; N 4.70%.

4.1.6. Ethyl 7-carbamoyl-4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylate (**6**)

4.1.6.1. Method a. Concentrated sulfuric acid (10.5 mL) was added to compound **5** (3.00 g, 10.3 mmol). The mixture was heated to 60 °C for 6 h. After cooling, ethyl acetate (100 mL) was added and the solution was neutralized with a *saturated sodium bicarbonate* solution. The organic layer was then separated, dried over sodium sulfate and evaporated under reduced pressure to afford **6** (2.90, 91%) as a yellow powder.

4.1.6.2. Method b. To a stirred suspension of compound **5** (200 mg, 0.76 mmol) in acetonitrile (10 mL) at 0 °C under argon was added chlorosulfonyl isocyanate (72 μL, 0.83 mmol). The mixture was then warmed to room temperature for 2 h and the reaction mixture was quenched with 1 M HCl (3 mL). After 1 h at room temperature, the precipitate was filtered and washed with water to afford **6** (172 mg, 74%) as a white powder.

R_f 0.61 (CH_2Cl_2); Mp: 202.5 °C; IR (KBr) ν 3430, 3387, 2982, 2939, 1699, 1644, 1601, 1457, 1380, 1319, 1282, 1215, 1141, 804, 767 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.11 (bs, 1H, $-N\underline{H}_2$), 6.34 (s, 1H, H_{ar}), 5.70 (bs, 1H, $-N\underline{H}_2$), 4.39 (q, 2H, J = 7.1 Hz, $-C\underline{H}_2CH_3$), 4.01 (s, 3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 2.68 (s, 3H, 3$-CH_3$), 1.41 (t, 3H, J = 7.1 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 160.4, 160.2, 158.5, 155.2, 140.1, 126.2, 113.8, 101.2, 90.5, 60.9, 57.1, 55.7, 14.4, 11.0; MS (ESI) m/z (%): 308.0 $[M + H]^+$. Anal. Calcd for $C_{15}H_{17}NO_6$: C 58.63; H 5.58; N 4.56. Found: C 59.02; H 5.72; N 4.60%.

4.1.7. 7-Carbamoyl-4,6-dimethoxy-3-methyl-1-benzofuran-2-carboxylic acid (**7**)

To a stirred solution of compound **6** (2.10 g, 6.83 mmol) in ethanol (15 mL) was added aqueous 1 M NaOH (6.8 mL, 6.83 mmol). The reaction mixture was refluxed for 4 h. After cooling, ethanol was removed under reduced pressure and the residue was precipitated with 1 M HCl. The suspension was then filtered to afford **7** (1.50 g, 79%) as a yellow powder.

R_f 0.61 (CH_2Cl_2/EtOH 9:1); Mp: 255.3 °C; IR (KBr) ν 3461, 3314, 2951, 1847, 1675, 1598, 1568, 1319, 1282, 1221, 1163, 1147, 976, 792, 712, 605 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 13.21 (bs, 1H, $-CO_2H$), 7.63 (bs, 1H, $-NH_2$), 7.48 (bs, 1H, $-NH_2$), 6.63 (s, 1H, H_{ar}), 4.00 (s, 3H, $-OCH_3$), 3.94 (s, 3H, $-OCH_3$), 2.64 (s, 3H, 3$-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 160.8, 157.8, 156.4, 152.7, 139.6, 124.9, 111.9, 104.4, 91.4, 56.7, 56.0, 11.0; MS (ESI) m/z (%): 280.0 $[M + H]^+$. Anal. Calcd for $C_{13}H_{13}NO_6$: C 55.92; H 4.69; N 5.02. Found: C 55.84; H 4.68; N 4.87%.

4.1.8. Synthesis of amides **8**–**9** using CNMPI procedure

*4.1.8.1. 4,6-Dimethoxy-3-methyl-N²-propyl-1-benzofuran-2,7-dicarboxamide (**8**).* Compound **7** (200.0 mg, 0.72 mmol) was suspended in dichloromethane (30 mL). 2-Chloro-1-methylpyridinium

iodide (183.0 mg, 0.72 mmol), amine (0.72 mmol) and triethylamine (251.6 μL, 1.79 mmol) were successively added and the mixture was refluxed for 10 h. The reaction mixture was diluted with dichloromethane (50 mL) and the organic layer was washed with water (3 × 30 mL), dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using CH_2Cl_2/EtOH 9:1 as eluent to give the desired product.

White powder. Yield 64% (147 mg). R_f 0.43 (CH_2Cl_2/EtOH, 9:1); Mp: 219 °C; IR (KBr) ν 3375, 3185, 2958, 2933, 1653, 1644, 1601, 1522, 1506, 1463, 1380, 1322, 1218, 1144, 1120, 801 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.96 (t, 1H, J = 6.0 Hz, −NH−), 7.62 (s, 1H, −NH_2), 7.42 (s, 1H, −NH_2), 6.62 (s, 1H, H_{ar}), 3.99 (s, 3H, −OCH_3), 3.93 (s, 3H, −OCH_3), 3.20–3.25 (m, 2H, −$C\underline{H}_2CH_2CH_3$), 2.62 (s, 3H, 3−CH_3), 1.52–1.58 (m, 2H, −$CH_2C\underline{H}_2CH_3$), 0.90 (t, 3H, J = 7.4 Hz, −$CH_2CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 159.2, 157.6, 156.2, 151.8, 141.7, 120.4, 112.2, 104.1, 91.6, 56.8, 56.0, 40.1, 22.5, 11.4, 10.5; MS (ESI) m/z (%): 321.1 $[M + H]^+$. Anal. Calcd for $C_{16}H_{20}N_2O_5$: C 59.99; H 6.29; N 8.74. Found: C 60.08; H 6.34; N 9.06%.

*4.1.8.2. N^2-Benzyl-4,6-dimethoxy-3-methylbenzofuran-2,7-dicarboxamide (**9**).* Compound **9** was obtained following the representative procedure described for compound **8**. White powder. Yield 69% (183 mg). R_f 0.77 (CH_2Cl_2); Mp: 278.8 °C; IR (KBr) ν 3369, 3172, 2933, 1650, 1604, 1509, 1466, 1430, 1384, 1325, 1261, 1218, 1163, 1144, 1123, 810, 693 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.54 (t, 1H, J = 6.2 Hz, −NH−), 7.59 (s, 1H, −NH_2), 7.37 (s, 1H, −NH_2), 7.32–7.33 (m, 4H, H_{benzyl}), 7.22–7.26 (m, 1H, H_{benzyl}), 6.59 (s, 1H, H_{ar}), 4.44 (d, 2H, J = 6.2 Hz, −CH_2−), 3.96 (s, 3H, −OCH_3), 3.90 (s, 3H, −OCH_3), 2.60 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 159.3, 157.7, 156.2, 151.8, 141.5, 139.6, 128.2 (2 C), 127.4 (2 C), 126.7, 121.1, 112.1, 104.1, 91.6, 56.8, 56.0, 41.9, 10.6; MS (ESI) m/z (%): 369.1 $[M + H]^+$. Anal. Calcd for $C_{20}H_{20}N_2O_5$: C 65.21; H 5.47; N 7.60. Found: C 65.47; H 5.55; N 7.81%.

*4.1.9. Synthesis of amides **10**–**12** using TBTU procedure*

*4.1.9.1. 4,6-Dimethoxy-3-methyl-N^2-phenyl-1-benzofuran-2,7-dicarboxamide (**10**).* To a stirred solution of compound **7** (100.0 mg, 0.36 mmol) in DMF (5 mL) were successively added *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (149.5 mg, 0.47 mmol), *N*,*N*-diisopropylethylamine (124.8 μL, 0.72 mmol) and amine (0.47 mmol). The reaction mixture was stirred at room temperature for 24 h. Water (30 mL) and dichloromethane (30 mL) were then added and the aqueous layer was extracted with dichloromethane (2 X 30 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using CH_2Cl_2/EtOH 95:5 as eluent to give the desired product.

White powder. Yield 90% (115 mg). R_f 0.77 (CH_2Cl_2/EtOH, 9:1); Mp: 237.7 °C; IR (KBr) ν 3369, 3191, 2921, 1684, 1635, 1540, 1442, 1316, 1255, 1215, 1144, 1120, 979, 807, 749, 687 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H, −NH−), 7.71 (d, 2H, J = 7.6 Hz, $H_{2\ and\ 6\text{-}phenyl}$), 7.63 (s, 1H, −$NH_2$), 7.45 (s, 1H, −$NH_2$), 7.35 (dd, 2H, J = 7.4 and J = 7.6 Hz, $H_{3\ and\ 5\text{-}phenyl}$), 7.11 (t, 1H, J = 7.4 Hz, $H_{4\text{-}phenyl}$), 6.63 (s, 1H, H_{ar}), 3.98 (s, 3 H, −OCH_3), 3.92 (s, 3H, −OCH_3), 2.63 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.0, 158.2, 157.8, 156.4, 152.1, 141.5, 138.2, 128.6 (2 C), 123.8, 122.1, 120.5 (2 C), 112.2, 103.9, 91.8, 56.8, 56.1, 10.7; MS (ESI) m/z (%): 355.1 $[M + H]^+$. Anal. Calcd for $C_{19}H_{18}N_2O_5$: C 64.40; H 5.12; N 7.91. Found: C 64.48; H 5.21; N 7.84%.

*4.1.9.2. 4,6-Dimethoxy-3-methyl-N^2-pyridin-2-yl-1-benzofuran-2,7-dicarboxamide (**11**).* Compound **11**was obtained following the representative procedure described for compound **10**. Beige powder. Yield 55% (70 mg). R_f 0.66 (CH_2Cl_2/EtOH, 9:1); Mp: 259.7 °C; IR (KBr) ν 3401, 3319, 2916, 1649, 1634, 1562, 1470, 1429, 1306, 1250, 1069, 841, 787 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.66 (s, 1H, −NH−), 8.37 (dd, 1H, J = 4.6 and 1.4 Hz, $H_{3\text{-}pyridine}$), 8.18 (d, 1H, J = 8.2 Hz, $H_{6\text{-}pyridine}$), 7.86 (ddd, 1H, J = 8.2, 7.4 and 1.4 Hz, $H_{5\text{-}pyridine}$), 7.75 (s, 1H, −NH_2), 7.50 (s, 1H, −NH_2), 7.18 (dd, 1H, J = 7.4 and 4.6 Hz, $H_{4\text{-}pyridine}$), 6.64 (s, 1H, H_{ar}), 3.99 (s, 3H, −OCH_3), 3.93 (s, 3H, −OCH_3), 2.66 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.8, 158.9, 157.7, 156.8, 152.3, 151.0, 148.2, 140.5, 138.5, 123.6, 120.0, 113.6, 112.2, 103.5, 92.1, 56.9, 56.2, 10.7; MS (ESI) m/z (%): 356.2 $[M + H]^+$. Anal. Calcd for $C_{18}H_{17}N_3O_5$: C 60.84; H 4.82; N 11.82. Found: C 60.67; H 4.80; N 11.89%.

*4.1.9.3. 4,6-Dimethoxy-3-methyl-N^2-pyridin-4-yl-1-benzofuran-2,7-dicarboxamide (**12**).* Compound **12**was obtained following the representative procedure described for compound **10**. Yellow powder. Yield 58% (74 mg). R_f 0.48 (CH_2Cl_2/EtOH, 9:1); Mp: 235.8 °C; IR (KBr) ν 3395, 3358, 2922, 1682, 1587, 1504, 1319, 1283, 1221, 1136, 1119, 1055 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.91 (s, 1H, −NH−), 8.57 (d, 2H, J = 5.5 Hz, $H_{3\ and\ 5\text{-}pyridine}$), 7.95 (d, 2H, J = 5.5 Hz, $H_{2\ and\ 6\text{-}pyridine}$), 7.69 (s, 1H, −$NH_2$), 7.50 (s, 1H, −$NH_2$), 6.63 (s, 1H, H_{ar}), 3.99 (s, 3H, −OCH_3), 3.91 (s, 3H, −OCH_3), 2.65 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.0, 158.7, 158.6, 156.6, 152.4, 148.2, 147.1 (2 C), 140.5, 124.8, 114.5 (2 C), 112.1, 103.8, 91.8, 56.8, 56.1, 10.8; MS (ESI) m/z (%): 356.1 $[M + H]^+$. Anal. Calcd for $C_{18}H_{17}N_3O_5$: C 60.84; H 4.82; N 11.82. Found: C 60.80; H 4.67; N 12.02%.

*4.1.10. 4,6-Dimethoxy-3-methyl-1-benzofuran-2,7-dicarboxamide (**13**)*

A solution of ammonia (7 N in methanol) was added to compound **6** (400 mg, 1.3 mmol) in a sealed tube and kept to reflux condition for 60 h. Solvent was then evaporated and the powder obtained was triturated with methanol and filtered. The crude product was purified by silica gel chromatography using CH_2Cl_2/EtOH 99:1 as eluent to give **13** (244 mg, 67%) as a beige powder.

R_f 0.49 (CH_2Cl_2/EtOH, 9:1); Mp: 271.1 °C; IR (KBr) ν 3420, 3345, 2922, 1659, 1607, 1566, 1391, 1310, 1219, 1171, 1142, 1113 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.60 (bs, 1H, −NH_2), 7.55 (bs, 1H, −NH_2), 7.42 (bs, 1H, −NH_2), 7.32 (bs, 1H, −NH_2), 6.62 (s, 1H, H_{ar}), 3.99 (s, 3H, −OCH_3), 3.93 (s, 3H, −OCH_3), 2.62 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 161.1, 157.8, 156.3, 151.8, 141.7, 121.0, 112.2, 103.9, 91.6, 56.8, 56.0, 10.6; MS (ESI) m/z (%): 279.1 $[M + H]^+$. Anal. Calcd for $C_{13}H_{14}N_2O_5$: C 56.11; H 5.07; N 10.07. Found: C 55.93; H 5.12; N 9.81%.

*4.1.11. (4,6-Dimethoxy-3-methylbenzofuran-2-yl)methanol (**14**)*

To a stirred suspension of lithium aluminum hydride (0.86 g, 22.7 mmol) in anhydrous THF (200 mL) was added dropwise a solution of compound **3** (5.00 g, 18.9 mmol) in anhydrous THF (100 mL) at room temperature under nitrogen atmosphere. The mixture was stirred at room temperature for 15 min and quenched with aqueous saturated $NaHCO_3$ solution (300 mL). The aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure to afford **14** (3.99 g, 95%) as a white powder.

R_f 0.16 (CH_2Cl_2); Mp: 109.2 °C; IR (KBr) ν 3337, 3240, 2992, 2933, 1623, 1601, 1507, 1454, 1417, 1389, 1319, 1262, 1216, 1198, 1155, 1112, 996, 812, 792 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.55 (d, 1H, J = 1.9 Hz, H_{ar}), 6.26 (d, 1H, J = 1.9 Hz, H_{ar}), 4.67 (d, 2H, J = 5.5 Hz, −$C\underline{H}_2OH$), 3.85 (s, 3H, −OCH_3), 3.83 (s, 3H, −OCH_3), 2.33 (s, 3H, 3−CH_3), 1.69 (t, 1H, J = 5.5 Hz, −OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2, 156.2, 155.3, 148.8, 113.4, 112.7, 93.9, 88.0, 55.7, 55.5, 55.4, 9.8; MS (ESI) m/z (%): 205.0 $[M + H-H_2O]^+$. Anal. Calcd for $C_{12}H_{14}O_4$: C 64.85; H 6.35. Found: C 64.86; H 6.01%.

4.1.12. 4,6-Dimethoxy-3-methyl-1-benzofuran-2-carbaldehyde (**15**)

To a stirred solution of compound **14** (3.50 g, 15.7 mmol) in distilled dichloromethane (100 mL) was added activated manganese(IV) oxide (8.22 g, 94.5 mmol). The mixture was refluxed for 2 h. The reaction mixture was filtered on Celite® and washed with dichloromethane. The organic filtrate was evaporated in vacuo to give **15** (3.40 g, 98%) as a yellow powder.

R_f 0.37 (CH_2Cl_2); Mp: 154.3 °C; IR (KBr) ν 2964, 2927, 2841, 1672, 1623, 1601, 1500, 1451, 1319, 1261, 1221, 1147, 1117, 1098, 868, 813, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.81 (s, 1H, −CHO), 6.55 (d, 1H, J = 1.6 Hz, H_{ar}), 6.25 (d, 1H, J = 1.6 Hz, H_{ar}), 3.88 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-OCH_3$), 2.66 (s, 3H, $3-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 163.3, 158.1, 157.3, 147.2, 112.8, 95.1, 87.7, 77.2, 55.8, 55.6, 10.1; MS (ESI) m/z (%): 221.0 $[M + H]^+$. Anal. Calcd for $C_{12}H_{12}O_4$: C 65.45; H 5.49. Found: C 65.30; H 5.72%.

4.1.13. 7-Bromo-4,6-dimethoxy-3-methyl-1-benzofuran-2-carbaldehyde (**16**)

Compound **16** was obtained following the same procedure as described for compound **4**. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **16** (1.26 g, 79%) as a yellow powder.

R_f 0.41 (CH_2Cl_2); Mp: 180–181 °C; IR (KBr) ν 3075, 2926, 1661, 1612, 1587, 1319, 1258, 1217, 1126, 1094, 826, 793 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H, −CHO), 6.71 (s, 1H, H_{ar}), 4.02 (s, 3H, $-OCH_3$), 4.01 (s, 3H, $-OCH_3$), 2.65 (s, 3H, $3-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.5, 158.5, 156.7, 153.8, 147.2, 131.0, 112.5, 93.0, 83.2, 57.4, 56.6, 10.0; MS (ESI) m/z (%): 299.0 $[M + H]^+$, 301.0 $[M + H+2]^+$. Anal. Calcd for $C_{12}H_{11}BrO_4$: C 48.18; H 3.71. Found: C 48.07; H 3.93%.

4.1.14. 2-Formyl-4,6-dimethoxy-3-methyl-1-benzofuran-7-carbonitrile (**17**)

Compound **17** was obtained following the same procedure as described for compound **5**. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **17** (732 mg, 50%) as an orange powder.

R_f 0.34 (cyclohexane/ethyl acetate 1:1); Mp: 263.9 °C; IR (KBr) ν 2227, 1653, 1617, 1595, 1506, 1436, 1322, 1261, 1224, 1172, 1123, 970, 810 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.88 (s, 1H, −CHO), 6.74 (s, 1H, H_{ar}), 4.07 (2 × s, 2 × 3H, 2 × $-OCH_3$), 2.63 (s, 3H, $3-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.4, 165.4, 161.4, 156.3, 147.5, 112.8, 112.0, 103.2, 92.1, 77.2, 57.6, 57.1, 9.6; MS (ESI) m/z (%): 246.0 $[M + H]^+$. Anal. Calcd for $C_{13}H_{11}NO_4$: C 63.67; H 4.52; N 5.71. Found: C 63.98; H 4.60; N 5.79%.

4.1.15. 2-Formyl-4,6-dimethoxy-3-methyl-1-benzofuran-7-carboxamide (**18**)

Compound **18** was obtained following the same procedure as described for compound **6**. The crude product was purified by silica gel chromatography using dichloromethane/EtOH 9:1 as eluent to afford **18** (296 mg, 44%) as a yellowish powder.

R_f 0.49 (CH_2Cl_2/EtOH 9:1); Mp: 260.1 °C; IR (KBr) ν 3381, 3185, 2927, 1727, 1653, 1607, 1571, 1316, 1261, 1218, 1144, 1120, 816 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.84 (s, 1H, −CHO), 7.63 (s, 1H, $-NH_2$), 7.50 (s, 1H, $-NH_2$), 6.63 (s, 1H, H_{ar}), 3.99 (s, 3H, $-OCH_3$), 3.93 (s, 3H, $-OCH_3$), 2.64 (s, 3H, $3-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.2, 164.1, 159.3, 157.4, 154.0, 147.1, 127.5, 111.7, 104.3, 91.7, 56.8, 56.3, 9.8; MS (ESI) m/z (%): 264.0 $[M + H]^+$. Anal. Calcd for $C_{13}H_{13}NO_5$: C 59.31; H 4.98; N 5.32. Found: C 59.34; H 4.86; N 5.44%.

4.1.16. 2-Formyl-4,6-dihydroxy-3-methyl-1-benzofuran-7-carboxamide (**21**)

To a stirred solution of compound **18** (80 mg, 0.3 mmol) in dry dichloromethane (6 mL) stirring under argon at −10 °C was added dropwise boron tribromide (1 M in CH_2Cl_2, 3.0 mL, 3.0 mmol). The mixture was then refluxed for 10 h. The reaction mixture was diluted with water and EtOAc. The organic phase was separated, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using dichloromethane/EtOH 19:1 as eluent to afford **21** (15 mg, 21%) as a beige powder.

R_f 0.24 (CH_2Cl_2/EtOH, 19:1); Mp > 360 °C; IR (KBr) ν 3454, 3177, 1659, 1612, 1393, 1327, 1238, 1190, 827 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 14.14 (bs, 1H, −OH), 11.54 (bs, 1H, −OH), 9.92 (s, 1H, −CHO), 8.36 (bs, 1H, $-NH_2$), 7.44 (bs, 1H, $-NH_2$), 6.25 (s, 1H, H_{ar}), 2.68 (s, 3H, $3-CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.9, 171.0, 168.0, 160.7, 156.0, 147.9, 131.7, 111.7, 99.8, 93.3, 10.8; MS (ESI) m/z (%): 236.0 $[M + H]^+$. Anal. Calcd for $C_{11}H_9NO_5$: C 56.18; H 3.86; N 5.96. Found: C 55.89; H 3.70; N 6.22%.

4.1.17. Phloroglucinol triacetate (**22**)

To a solution of phloroglucinol (10.0 g, 79.2 mmol) in pyridine (50 mL) was added acetic anhydride (30 mL, 317.2 mmol). The mixture was stirred and refluxed for 4 h. Pyridine was then removed under reduced pressure and the residue was washed with diisopropyl ether to give **22** (19.6 g, 98%) as a white powder.

R_f 0.23 (cyclohexane/EtOAc 5:1); Mp: 106.6 °C (litt [32]. 106–107 °C); IR (KBr) ν 3105, 1757, 1601, 1366, 1182, 1121, 1018, 914 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.84 (s, 3H, H_{ar}), 2.28 (s, 9H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7 (C═O), 151.2 (C_{ar}—O), 112.9 (C_{ar}—H), 21.2 ($-CH_3$); MS (ESI) m/z (%): 251.1 $[M - H]^-$.

4.1.18. Phloroglucinol tribenzyl ether (**23**)

A mixture of phloroglucinol triacetate **22** (3.0 g, 11.9 mmol), benzyl chloride (4.93 mL, 42.8 mmol) and sodium hydride (60% in mineral oil) (3.43 g, 85.7 mmol) in DMF (60 mL) was cooled to 0 °C and water (0.64 mL, 35.7 mmol) was added dropwise over a period of 10 min. The reaction mixture was stirred overnight at room temperature poured into water. The aqueous layer was extracted with ethyl acetate and organic layer was washed with brine, dried over Na_2SO_4 and filtered. The solvent was evaporated in vacuo and the residue was recrystallized from methanol to give **23** (3.12 g, 78%) as a white powder.

R_f 0.66 (cyclohexane/EtOAc 4:1); Mp: 88.5 °C (lit [33]. 93.0–94.0 °C); IR (KBr) ν 2922, 1593, 1449, 1377, 1155, 1063, 1026, 804, 727, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.33–7.43 (m, 15H, H_{Ar}), 6.28 (s, 3H, $H_{2,4,6}$), 5.01 (s, 6H, $-CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (3 C), 136.8 (3 C), 128.6 (6 CH), 128.0 (3 CH), 127.6 (6 CH), 94.9 (3 CH), 70.1 (3-CH_2); MS (ESI) m/z (%): 397.2 $[M + H]^+$.

4.1.19. 2,4,6-Tribenzyloxyacetophenone (**24**)

Acetic acid (2.2 mL, 38.4 mmol) and TFAA (6.4 mL, 45.3 mmol) were stirred for 5 min at room temperature. Phloroglucinol tribenzyl ether **23** (5.0 g, 12.6 mmol) was dissolved in CH_2Cl_2 (200 mL) and then added at 0 °C. After 1.5 h, the deep purple solution was diluted with AcOEt and poured into saturated aq. $NaHCO_3$ solution. The yellow organic layer was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using dichloromethane/cyclohexane 6:4 as eluent to afford **24** (4.1 g, 75%) as a yellowish oil.

R_f 0.43 (cyclohexane/EtOAc 4:1); IR (KBr) ν 3032, 2870, 1692, 1599, 1584, 1431, 1153, 1111, 735, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.26–7.39 (m, 15H, H_{Ar}), 6.24 (s, 2H, $H_{3,5}$), 5.04 (s, 4H, 2-CH_2), 4.99 (s, 2H, $-CH_2$), 2.47 (s, 3H, $-COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.6 (C═O), 161.1, 157.2 (2 C), 136.5 (2 C), 136.4, 128.7 (2 C), 128.6 (4 C), 128.2, 128.0 (2 C), 127.5 (2 C), 127.1 (4 C), 115.1, 93.5 (2 C), 70.6 (2 C), 70.3, 32.7 ($-CO\underline{C}H_3$); MS (ESI) m/z (%): 439.1 $[M + H]^+$.

*4.1.20. 2-Hydroxy-4,6-dibenzyloxyacetophenone (**25**)*

$TiCl_4$ (1 M in CH_2Cl_2, 6.3 mL, 6.3 mmol) was added dropwise at −5 °C to a stirred solution of **24** (4.6 g, 10.5 mmol) in CH_2Cl_2 (70 mL). After 1.5 h of stirring, the mixture was slowly poured into aqueous saturated $NaHCO_3$, washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using dichloromethane/cyclohexane 1:3 as eluent to afford **25** (3.1 g, 84%) as a white powder after trituration in methanol.

R_f 0.28 (cyclohexane/CH_2Cl_2 1:1); Mp: 102–104 °C (lit [21]. 104 °C); IR (KBr) ν 1614, 1587, 1265, 1171, 1103, 1080, 826, 727, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 14.03 (s, 1H, −OH), 7.26–7.41 (m, 10H, H_{Ar}), 6.16 (d, J = 2.3 Hz, 1H, H_{ar}), 6.10 (d, J = 2.3 Hz, 1H, H_{ar}), 5.06 (s_{app}, 4H, 2-CH_2), 2.55 (s, 3H, −$COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.2 (C=O), 167.6, 165.1, 162.0, 135.9, 135.6, 128.8 (2 C), 128.7 (2 C), 128.5, 128.4, 128.0 (2 C), 127.7 (2 C), 106.3, 94.7, 92.4, 71.1, 70.3, 33.3 (−CO$\underline{C}H_3$); MS (ESI) m/z (%): 349.2 $[M + H]^+$.

*4.1.21. Ethyl 4,6-dibenzyloxy-3-methyl-1-benzofuran-2-carboxylate (**26**) and 4,6-dibenzyloxy-3-methyl-1-benzofurane (**27**)*

To a stirred solution of compound **25** (3.34 g, 9.6 mmol) in DMF (20 mL) were successively added ethyl bromoacetate (1.17 mL, 10.5 mmol) and potassium carbonate (3.98 g, 28.8 mmol). The reaction mixture was heated to 70 °C for 2 h, then to 100 °C for 20 h. The reaction mixture was cooled to room temperature and filtered on Celite®. The solution was diluted with ethyl acetate (200 mL) and the organic layer was washed with brine and water, dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using cyclohexane/dichloromethane 4:1 as eluent to afford **26** (2.08 g, 52%) as a white powder and **27** (0.65 g, 20%) as a white powder.

*4.1.21.1. Ethyl 4,6-dibenzyloxy-3-methyl-1-benzofuran-2-carboxylate (**26**).* R_f 0.27 (cyclohexane/CH_2Cl_2, 4:1); Mp: 103–105 °C; IR (KBr) ν 1707, 1616, 1595, 1273, 1150, 1096, 1063, 1024, 806, 739, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34–7.44 (m, 10H, H_{benzyl}), 6.68 (d, 1H, J = 1.4 Hz, H_7), 6.45 (d, 1H, J = 1.4 Hz, H_5), 5.13 (s, 2H, −CH_2), 5.07 (s, 2H, −CH_2), 4.41 (q, 2H, J = 7.1 Hz, −$C\underline{H}_2CH_3$), 2.70 (s, 3H, 3−CH_3), 1.41 (t, 3H, J = 7.1 Hz, −$CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6, 160.5, 156.6, 155.5, 139.2, 136.4 (2 C), 128.7 (2 C), 128.6 (2 C), 128.2, 128.1, 127.6 (2 C), 127.2 (3 C), 113.2, 96.4, 89.4, 70.6, 70.2, 60.7, 14.5, 11.4; MS (ESI) m/z (%): 417.2 $[M + H]^+$. Anal. Calcd for $C_{26}H_{24}O_5$: C 74.98; H 5.81. Found: C 75.09; H 5.66%.

*4.1.21.2. 4,6-Dibenzyloxy-3-methyl-1-benzofurane (**27**).* R_f 0.92 (CH_2Cl_2); Mp: 132–134 °C; IR (KBr) ν 2924, 1616, 1497, 1452, 1377, 1314, 1148, 1117, 1094, 1065, 1024, 808, 737, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.32–7.40 (m, 10H, H_{benzyl}), 7.19 (d, 1H, J = 1.0 Hz, H_2), 6.68 (d, 1H, J = 1.8 Hz, H_7), 6.46 (d, 1H, J = 1.8 Hz, H_5), 5.12 (s, 2H, −CH_2), 5.07 (s, 2H, −CH_2), 2.34 (d, 3H, J = 1.0 Hz, 3−CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.9, 157.3, 154.1, 139.2, 137.0, 136.9, 128.6 (2 C), 128.5 (2 C), 128.0, 127.8, 127.6 (2 C), 127.1 (2 C), 115.8, 112.6, 95.6, 89.8, 70.6, 70.0, 10.0; MS (ESI) m/z (%): 345.1 $[M + H]^+$. Anal. Calcd for $C_{23}H_{20}O_3$: C 80.21; H 5.85. Found: C 80.60; H 5.98%.

*4.1.22. 4,6-Dibenzyloxy-3-methyl-1-benzofuran-2-carbaldehyde (**28**)*

Phosphorus oxychloride (145 μL, 1.55 mmol) and DMF (2.1 mL) were stirred at room temperature for 1 h under argon atmosphere. Compound **27** (465 mg, 1.35 mmol) was then added portionwise and the resulting mixture was stirred overnight, quenched with 8 N aqueous NaOH. The reaction mixture was extracted with ethyl acetate (50 mL) and the organic layer was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **28** (506 mg, 83%) as a white powder.

R_f 0.25 (CH_2Cl_2); Mp: 106–108 °C; IR (KBr) ν 2920, 1665, 1612, 1591, 1319, 1254, 1233, 1163, 1092, 733, 696, 660 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.84 (s, 1H, −CHO), 7.37–7.45 (m, 10H, H_{benzyl}), 6.66 (d, 1H, J = 1.8 Hz, H_7), 6.45 (d, 1H, J = 1.8 Hz, H_5), 5.14 (s, 2H, −CH_2), 5.09 (s, 2H, −CH_2), 2.69 (s, 3H, 3−CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 162.2, 158.0, 156.2, 147.3, 136.1, 128.7 (4 C), 128.3, 128.2, 127.6 (2 C), 127.3 (2 C), 113.1, 96.7, 89.3, 70.7, 70.4, 10.3; MS (ESI) m/z (%): 373.1 $[M + H]^+$. Anal. Calcd for $C_{24}H_{20}O_4$: C 77.40; H 5.41. Found: C 77.28; H 5.76%.

*4.1.23. General procedure of debenzylation − synthesis of compounds **29**, **30** and **32***

To a solution of 4,6-dibenzyloxybenzofurane derivative (0.24 mmol) in a mixed solution of MeOH (2.5 mL), THF (2.5 mL) and cyclohexene (1 mL) was added Pd/C 10% (102 mg, 0.10 mmol). The suspension was refluxed for 5 h (compound **29**) or for 2 h (compounds **30** and **32**). Pd/C was filtered on Celite®, and the filtrate was concentrated in vacuo. The crude product was purified by silica gel chromatography using dichloromethane as eluent to afford **29**, and was triturated in dichloromethane to obtain compounds **30** and **32**.

*4.1.23.1. Ethyl 4,6-dihydroxy-3-methyl-1-benzofuran-2-carboxylate (**29**).* Brown solid. Yield 92% (52 mg). R_f 0.16 (CH_2Cl_2/EtOH, 19:1); Mp: 220–223 °C; IR (KBr) ν 3329, 1680, 1620, 1329, 1169, 1152, 1082, 835, 627 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H, −OH), 9.82 (s, 1H, −OH), 6.39 (s, 1H, H_{ar}), 6.25 (s, 1H, H_{ar}), 4.31 (q, 2H, J = 7.1 Hz, −$C\underline{H}_2CH_3$), 2.63 (s, 3H, 3−CH_3), 1.34 (t, 3H, J = 7.1 Hz, −$CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.6 (2 C), 156.7, 154.5, 137.3, 126.8, 110.3, 98.1, 88.9, 60.1, 14.2, 10.9; MS (ESI) m/z (%): 237.1 $[M + H]^+$. Anal. Calcd for $C_{12}H_{12}O_5$: C 61.02; H 5.12. Found: C 60.74; H 5.15%.

*4.1.23.2. 4,6-Dihydroxy-3-methyl-1-benzofuran-2-carbaldehyde (**30**).* Brown powder. Yield 61% (26 mg). R_f 0.45 (CH_2Cl_2/EtOH, 19:1); Mp > 360 °C; IR (KBr) ν 3464, 1593, 1302, 1140, 1072, 818, 789 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.52 (bs, 1H, −OH), 10.10 (bs, 1H, −OH), 9.78 (s, 1H, −CHO), 6.38 (d, 1H, J = 1.7 Hz, H_{ar}), 6.26 (d, 1H, J = 1.7 Hz, H_{ar}), 2.65 (s, 3 H, 3−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.2, 161.2, 157.9, 155.6, 146.1, 131.6, 110.4, 98.3, 88.9, 9.6; MS (ESI) m/z (%): 193.1 $[M + H]^+$. Anal. Calcd for $C_{10}H_8O_4$: C 62.50; H 4.20. Found: C 62.80; H 4.16%.

*4.1.23.3. Ethyl 7-carbamoyl-4,6-dihydroxy-3-methyl-1-benzofuran-2-carboxylate (**32**).* Brown powder. Yield 61% (55 mg). R_f 0.61 (CH_2Cl_2/EtOH, 19:1); Mp: 296–297 °C; IR (KBr) ν 3458, 3140, 1659, 1614, 1331, 1244, 1169, 1009, 829, 766, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 13.80 (s, 1H, −OH), 11.39 (bs, 1H, −OH), 8.30 (bs, 1H, −NH), 7.14 (bs, 1H, −NH), 6.19 (s, 1H, H_5), 4.32 (q, 2H, J = 7.1 Hz, −$C\underline{H}_2CH_3$), 2.61 (s, 3H, 3−CH_3), 1.32 (t, 3H, J = 7.1 Hz, −$CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9, 165.5, 159.0 (2 C), 153.6, 138.1, 126.8, 110.3, 98.4, 92.0, 60.5, 14.2, 10.7; MS (ESI) m/z (%): 280.2 $[M + H]^+$. Anal. Calcd for $C_{13}H_{13}NO_6$: C 55.92; H 4.69; N 5.02. Found: C 56.13; H 4.61; N 5.04%.

*4.1.24. Ethyl 4,6-dibenzyloxy-7-carbamoyl-3-methyl-1-benzofuran-2-carboxylate (**31**)*

To a stirred suspension of compound **26** (1.50 g, 3.60 mmol) in acetonitrile (60 mL) at 0 °C under argon was added chlorosulfonyl isocyanate (495 μL, 5.68 mmol). The mixture was then warmed to room temperature for 5 h and the reaction mixture was quenched with 1 M HCl. After 1 h at room temperature, the precipitate was

filtered and washed with water to afford **31** (1.44 g, 87%) as a white powder.

R_f 0.26 (CH_2Cl_2/EtOH, 19:1); Mp: 177–179 °C; IR (KBr) ν 3385, 3211, 1701, 1632, 1611, 1275, 1138, 1113, 1067, 723, 692, 617 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.73 (bs, 1H, –NH), 7.36–7.56 (m, 11H, H_{benzyl} and –NH), 6.92 (s, 1H, H_5), 5.36 (s, 2H, $-CH_2$), 5.30 (s, 2H, $-CH_2$), 4.36 (q, 2H, J = 7.1 Hz, $-C\underline{H}_2CH_3$), 2.68 (s, 3H, 3–CH_3), 1.35 (t, 3H, J = 7.1 Hz, $-CH_2C\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 159.3, 156.8, 155.1, 152.7, 138.9, 136.7, 136.3, 128.6 (2 C), 128.4 (2 C), 128.0, 127.8, 127.5 (4 C), 125.8, 112.3, 105.5, 94.4, 70.8, 70.0, 60.5, 14.2, 11.0; MS (ESI) m/z (%): 460.1 $[M + H]^+$. Anal. Calcd for $C_{27}H_{25}NO_6$: C 70.58; H 5.48; N 3.05. Found: C 70.69; H 5.55; N 2.92%.

4.2. Biological evaluation

4.2.1. Cytotoxicity activity assays (NSCLC-N6 and A549)

The antiproliferative activity of compounds **3-6**, **8**–**13**, **15**–**18**, **21**, **26**, **28**–**32** was evaluated. The NSCLC-N6 cell line [26], derived from a human non-small-cell bronchopulmonary carcinoma (moderately differentiated, rarely keratinized, classified as T2N0M0), and A549 obtained from ATCC collection reference CCL-185 [27] were used for all experiments. Both cell lines were cultured in RPMI 1640 medium with 5% fetal calf serum, to which were added 100 IU penicillin.mL^{-1}, 100 μg streptomycin.mL^{-1} and 2 mM glutamine, at 37 °C in an air/carbon dioxide atmosphere (95:5, v/v). Cytotoxicity was determined by continuous drug exposure. Experiments were performed in 96 wells microtiter plates (10^5 cells mL^{-1} for NSCLC-N6 and 2×10^{-4} cells mL^{-1} for A549). Cell growth was estimated by a colorimetric assay based on the conservation of tetrazolium dye (MTT) to a blue formazan product by live mitochondria [34]. Eight repeats were performed for each concentration. Control growth was estimated from 8 determinations. Optical density at 570 nm corresponding to solubilized formazan was read for each well on a Titertek Multiskan MKII.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.013.

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