European Journal of Medicinal Chemistry 74 (2014) 41–49

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Benzofuran derivatives as a novel class of inhibitors of mTOR signaling

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ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form
20 December 2013
Accepted 21 December 2013
Available online 2 January 2014

Keywords:
Benzofuran
Cytotoxicity
mTOR
mTORC1

ABSTRACT

High-throughput screening (HTS) hit **1** was previously identified as an inhibitor of the Akt/mTOR (Akt/mammalian target of rapamycin) signaling, which is a major target in oncology. The cytotoxicity of **1** was determined on a panel of human cancer cells lines with an IC_{50} comprised between 30 and 140 μM. Subsequent structure–activity relationship (SAR) studies led us to the identification of compounds that displayed an enhanced cytotoxicity. We demonstrated also that these molecules directly bind to mTOR complex 1 (mTORC1) and inhibit its kinase activity.

1. Introduction

The AKT/mammalian target of rapamycin (mTOR) pathway is considered as one of the most commonly activated and deregulated signaling pathways in human cancer [1–3]. Rapamycin (sirolimus), a naturally occurring macrolide, was the first identified potent inhibitor of mTOR. This kinase is associated with other proteins in two molecular complexes: mTORC1 and mTORC2. Rapamycin binds to the cytosolic immunophilin protein FKBP12 (FK-binding protein 12), which selectively inhibits mTORC1, without interacting with mTORC2. Several rapamycin derivatives (rapalogues) such as temsirolimus, everolimus and deforolimus, are currently commercialized to treat cancers. Temsirolimus and everolimus have been approved for the first- and second-line treatment, respectively, of patients with clear renal cell carcinomas. Moreover, temsirolimus was also approved for the treatment of mantle cell lymphoma.

Current clinical development of rapalogues was impaired by toxicity when combined with chemotherapy and several targeted therapies [1–3]. Furthermore, the antiproliferative activity of rapalogues appears to be limited by various molecular mechanisms of resistance in cancer cells combined to a lack of mTORC2 complex inhibition.

Novel anticancer drugs designed to overcome potential mechanisms of resistance to rapalogues are currently in development [1]. Several companies have identified drugs that inhibit mTOR by competing with adenosine triphosphate (ATP) into the ATP-binding sites. These compounds do not require FKBP12 to bind with mTOR and, thereby, inhibit both mTORC1 and mTORC2 complexes. Some of these molecules do not only inhibit mTOR, but also phosphatidylinositide 3-kinases (PI3K). Several specific mTOR inhibitors and dual PI3K/mTOR inhibitors are currently investigated in phase III trials.

Compound **1** (also known as ChemBridge 5219657, Fig. 1) was identified in a high-throughput screen as an inhibitor of Akt/mTOR signaling [4,5]. In 786-O renal carcinoma cells, **1** blocked Akt/mTOR-induced nuclear export of the transcription factor FOXO1a (IC_{50} = 20 μM) and cell proliferation (IC_{50} = 10 μM) by an unidentified mechanism downstream of Akt. The privileged structure of benzofurans, which is uncommon among the known inhibitors of the Akt/mTOR cascade, prompted us to define the structural requirements of **1** for its antiproliferative effects and to examine the pharmacological potential of this new class of cytostatic agents. Herein, we report the first SAR study on **1** and our efforts to identify its mechanism of action.

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http://dx.doi.org/10.1016/j.ejmech.2013.12.020

2. Results and discussions

2.1. Chemical synthesis

The synthesis of 2-benzyl-benzofuran derivatives started from the condensation of phenol **2** with chloroacetophenone **3** to afford the 2-benzoyl-5-methoxybenzofurane **4** [6]. Subsequent reduction of **4** by $NaBH_3CN$ and TMSCl [7] and demethylation by BBr_3 afforded the key intermediate **6** (Scheme 1). A Mannich reaction on phenol **6** with various secondary amines afforded the expected adducts **7**. A Mannich condensation was also performed on phenol **8** obtained by demethylation of **4**, to generate **10**, which upon a reduction with $NaBH_4$ afforded the alcohol **11**.

The synthesis of 2-phenyl-benzofurane **13a** was performed, in one step, from benzoquinone as described by Lyubchanskaya [8] (Scheme 2). Next, a Mannich reaction with amines **9a**–**b** afforded, as previously, the expected adducts **13a**–**b**. To detect, by pull-down experiments, the molecular target of these compounds, we saponified **13b** and conjugated the cognate carboxylic acid with an amino linker using ECDI/HOBT in CH_2Cl_2 at 0 °C to afford the azide **13c** that was hydrogenated to the amine **13d** and conjugated to Affi-Gel 10.

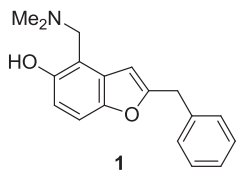
2.2. Biological results

2.2.1. In vitro cytotoxicity

First, we examined the antiproliferative effects of **1** on a panel of human colon (HT29, HCT116, Colo205, HCC2998), ovarian (OVCAR3, IGROV1), lung (HOP62, HOP92), breast (MCG7), melanoma (MDA-MB-231), prostate (DU145) and head and neck (SQ20B) cancer cell lines to verify its cytotoxicity and to identify a cell line suitable to assay its derivatives and analogs (Fig. 2). HCC2998, SQ20B and HOP92 cell lines were found to be particularly sensitive to **1** cytotoxicity ($IC_{50} \approx 40$ μM).

Based on these data, the head and neck cancer cell line SQ20B was chosen to test the cytotoxicity of synthesized compounds. These cells have a constitutively active mutation in epidermal growth factor receptor (EGFR) resulting in a constitutive activation of Akt. They are used as a cellular model of radioresistant cancers that are weakly sensitive to cisplatine.

Our SAR study began by investigating the effects of substitutions on the amine moiety (Table 1). Replacement of the dimethylamino moiety of **1** by a bulkier and more hydrophobic diethylamino **7a**, *N*-methyl-cyclohexylamino (**7b**), tetrahydroisoquinolino (**7c**) or *N*-methyl-benzylamino (**7d**) slightly increased cytotoxicity. Substitution by a *N*,*O*-dimethylhydroxylamino group (**7e**) was well tolerated, but introduction of a hydroxyl (**7f**–**h**) led to a significant loss of potency. Inclusion of the amine in a morpholine ring (**7i**) slightly increased the activity, and the insertion in *N*-substituted basic piperazine rings was well allowed (**7j**–**m**). Interestingly, hydroxyl-piperidino (**7n**, **o**) derivatives were as active as diethylamine **7a**. Introduction of a carboxamide (**7p**) or a phenyl group (**7q**) in the position 4 of the piperidino moiety was detrimental. Gratifyingly, a major improvement in potency resulted from the substitution by a 4-piperidinopiperidine (**7r**) or a 4-pyrrolidinopiperidine moiety (**7s**).



1

Fig. 1. Structure of HTS hit **1** [4].

Compound **7r** was then selected as the starting point for the design of an affinity ligand to identify the molecular target of this class of compounds by pull-down assays. En route to this objective, we synthesized **7t** and demonstrated that the introduction of a linker on the terminal piperidine moiety was well tolerated for the cytotoxicity. In the meanwhile, we examined the influence of the benzyl moiety. Functionalization of the benzylic position as a ketone (**10**) or an alcohol (**11**) maintained the cytotoxicity almost unaltered. Gratifyingly, the deletion of this benzylic methylene (**13a**) conserved the cytotoxicity. We combined these data to design the affinity probe **13e** (Scheme 2) that entails a 2-phenylbenzofuran substituted by a linker based on **7t**.

Western blot analysis of pull-down assays gave positive results with antibodies targeting mTOR but not other proteins that belong to the same signaling pathway, such as Akt, Rictor, Raptor, PI3K and PDK1 (pyruvate dehydrogenase lipoamide kinase isozyme 1) (Fig. 3a). No unspecific binding was observed with Affi-Gel 10 beads containing only the linker as a negative control. Our lead compounds **7s** and **13e** were shown to inhibit in a dose dependent manner the phosphorylation of S6 ribosomal protein, which is a target of the mTOR complex 1 (mTORC1) (Fig. 3b and c). In addition, the phosphorylation of AKT (Ser473) was increased after exposure to high doses of **13e**, suggesting a specific inhibition of mTORC1 [9].

In comparison with clinical mTOR inhibitor, **7r** and **13a** displayed a cytotoxicity in the same range of concentration than everolimus and OZI027 [10] on a panel of human cancer cell lines, but with a different profile of selectivity (Table 2), suggesting that this new class of anticancer agents may find clinical applications that are complementary to those of everolimus and OZI027.

3. Conclusion

In conclusion, we performed the first hit to lead optimization of **1** and demonstrated that the replacement of the dimethylamine and benzyl moieties by a 4-piperidino-piperidine and a phenyl significantly improves cytotoxicity. We also demonstrated that this new class of compounds interacts with the complex mTORC1 to inhibit its activity. The optimization of **13a** is currently under investigation to increase its antiproliferative potency.

4. Experimental protocol

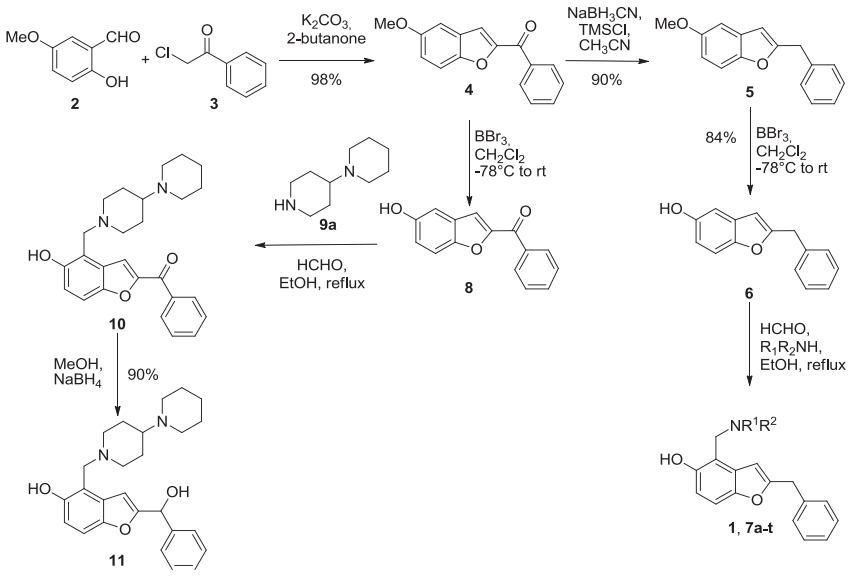
4.1. Biology

4.1.1. Cell lines

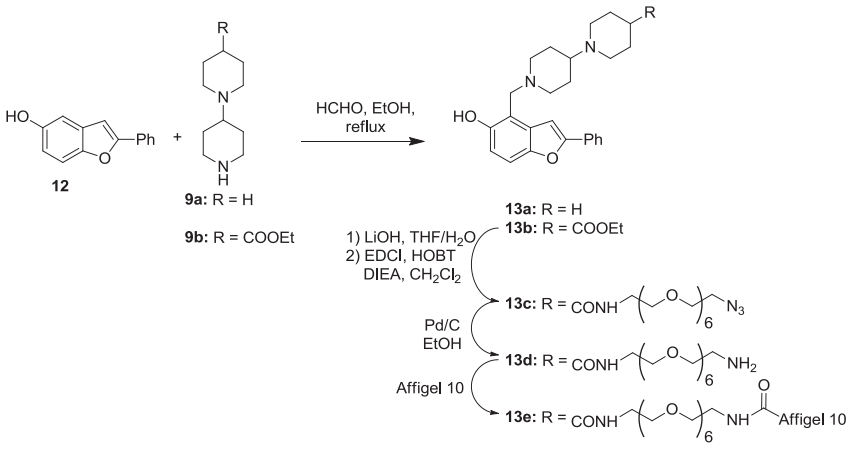
Hepatocarcinoma SK-HEP1, HEPG2, prostate DU145, renal CAKI1 and ovarian OVCAR3 cell lines were obtained from the ATCC (Rockville, MD). Colon Colo205, HCT116, HT29, and HSCLC HOP62 cell lines were purchased from NCI cell line bank. SQ20B was kindly provided by Prof. E. Deutsch (Institute Gustave Roussy, France). ColoR was developed in our laboratory. Cells were grown as monolayers in RPMI medium supplemented with 10% fetal calf serum (Invitrogen, Cergy-Pontoise, France), 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified 5% CO_2 atmosphere, and regularly checked for the absence of *Mycoplasma*.

4.1.2. Cell cytotoxicity assay

Cell survival was determined using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, Saint-Quentin Fallavier, France). The conversion of yellow water-soluble tetrazolium MTT into purple insoluble formazan is catalyzed by mitochondrial dehydrogenases and used to estimate the number of viable cells. In brief, cells were seeded in 96-well tissue culture plates at a density of 2×10^3 cells/well. After drug exposure,



Scheme 1. Synthesis of **1** and its derivatives **7a**–**t**, **10** and **11**.



Scheme 2. Synthesis of 2-phenylbenzofuran derivatives **13a**–**e**.

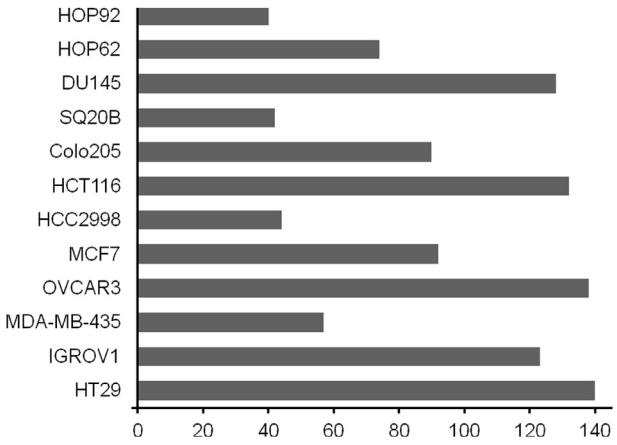


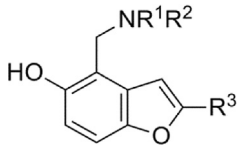
Fig. 2. Inhibition of cell proliferation by **1** on various cancer cell lines (IC_{50}, μM). IC_{50} values were determined from dose–response curves of viability assessed by MTT assay after a 72 h treatment according to the method described under Experimental section.

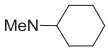
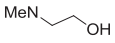
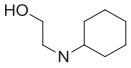
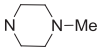
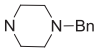
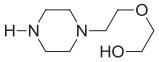
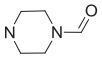
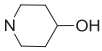
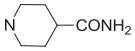
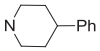
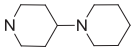
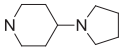
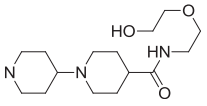
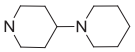
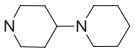
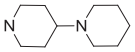
cells were incubated with 0.4 mg/ml MTT for 4 h at 37 °C. After incubation, the supernatant was discarded, insoluble formazan precipitates were dissolved in 0.1 mL of DMSO and the absorbance was measured at 560 nm by use of a microplate reader (Thermo, France). Wells with untreated cells or with drug-containing medium without cells were used as positive and negative controls respectively. Everolimus and OZI027 were purchased from Selleck Chemicals (Munich, Germany).

4.1.3. Western blot analysis

Cells were lysed in buffer containing 50 mM HEPES (pH 7.6), 150 mM NaCl, 1% Triton X-100, 2 mM sodium vanadate, 100 mM NaF, and 0.4 mg/ml phenylmethylsulfonyl fluoride. Equal amounts of protein (30 μg/lane) were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% milk in 0.05% Tween 20/phosphate-buffered saline and then incubated with the primary antibody overnight. Membranes were then washed and incubated with the secondary antibody conjugated to horseradish peroxidase. Bands were visualized by using the enhanced chemiluminescence Western blotting detection system. Densitometric analysis was performed under conditions that yielded a linear response.

Table 1
Cytotoxicity of benzofuran derivatives against SQ20B human cancer cell line (IC_{50}, μM).[a]



Cpd	NR^1R^2	R^3	IC_{50} (μM)
1	NMe_2	CH_2Ph	33 ± 2.8
7a	NEt_2	CH_2Ph	12 ± 4.2
7b		CH_2Ph	12 ± 2.2
7c		CH_2Ph	16 ± 0.6
7d		CH_2Ph	12 ± 5.0
7e	NMeOMe	CH_2Ph	40 ± 7.4
7f		CH_2Ph	30 ± 5.7
7g		CH_2Ph	27 ± 6.2
7h	$N(CH_2CH_2OH)_2$	CH_2Ph	>200
7i		CH_2Ph	6.5 ± 2.3
7j		CH_2Ph	18 ± 4.7
7k		CH_2Ph	36 ± 5.1
7l		CH_2Ph	21 ± 6.7
7m		CH_2Ph	49 ± 6.4
7n		CH_2Ph	11 ± 2.5
7o		CH_2Ph	13 ± 2.9
7p		CH_2Ph	85 ± 11
7q		CH_2Ph	>200
7r		CH_2Ph	3.0 ± 0.9
7s		CH_2Ph	3.0 ± 1.1
7t		CH_2Ph	14 ± 0.9
10		COPh	17 ± 1.6
11		CH(OH)Ph	13 ± 3.4
13a		Ph	2.8 ± 0.7

[a] Data are the average of two independent IC_{50} value determinations.

4.1.4. Pull-down assay

Sub-confluent SQ20B cells were collected and washed in phosphate-buffered saline 1× (PBS). Lysis was performed by freezing the cells in liquid N_2 in 500 μL of lysis buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, protease inhibitor cocktail 1×). Cellular debris was removed by centrifugation at 10,000 × *g* for 10 min. Five hundred microgram of total protein extract was incubated for 10–12 h at 4 °C with 40 μL of agarose conjugate **13e**. The beads were washed extensively with lysis buffer and bound-proteins were eluted by SDS sample buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 1.4 M β-mercaptoethanol, and bromophenol blue). Eluted proteins were recovered from the beads by centrifugation. Total proteins, unbound proteins, or eluted proteins were resolved by 12%–8% SDS-PAGE and electrotransferred to nitrocellulose sheets (Schleicher and Schuell, Dassel, Germany). The membrane was blocked in PBS containing 5% nonfat dry milk and 0.1% Tween 20 and then incubated with the primary antibody overnight. After washing, the blots were incubated with appropriate secondary antibodies. Horseradish peroxidase-conjugated (HRP) AffiniPure donkey anti-rabbit at 1/10000 (Jackson ImmunoResearch) and HRP donkey anti-goat at 1/1000 (Santa Cruz biotechnology, Santa Cruz, CA) were used. Finally, protein-antibody complexes were visualized by an enhanced chemiluminescence detection system (SuperSignal West Pico, Pierce).

4.2. Chemistry

All reagents and solvents for synthesis were purchased from Sigma–Aldrich, Fluka, or Acros and used without further purification. Column chromatography was carried out on silica gel 60 (Merck, 70–230 mesh). ^1H NMR spectra at 300 MHz or 400 MHz and ^{13}C NMR spectra at 75 MHz or 100 MHz were recorded with DPX 300 SY Brucker spectrometers with the deuterated solvent as the lock and residual solvent as the internal reference. All chemical shift values and coupling constants *J* are quoted in ppm and in Hz, respectively. Infrared spectra were recorded using a Perkin–Elmer 881. Analytical RP-HPLC-MS was performed using a C18 column (30 mm × 1 mm; 1.9 μm) using the following parameters: (1) the solvent system A (acetonitrile) and B (0.05% TFA in H_2O); (2) the linear gradient $t = 0$ min with 98% B, $t = 5$ min with 5% B, $t = 6$ min with 5% B, $t = 7$ min with 98% B, and $t = 9$ min with 98% B; (3) flow rate of 0.3 mL/min; (4) column temperature 50 °C; (5) ratio of products determined by integration of spectra recorded at 210 or 254 nm; (6) ionization mode ESI. Melting points (mp [°C]) were taken on samples in open capillary tubes.

4.2.1. General procedure for the Mannich reaction (method A)

The secondary amine (2 eq) was added to an EtOH (0.1–0.2 M) solution of 5-hydroxy-benzofuran derivative (1 eq) and formaldehyde (37% in water, 2 eq). The solution was stirred at reflux (3 h–16 h) and concentrated under vacuum. The residue was purified by

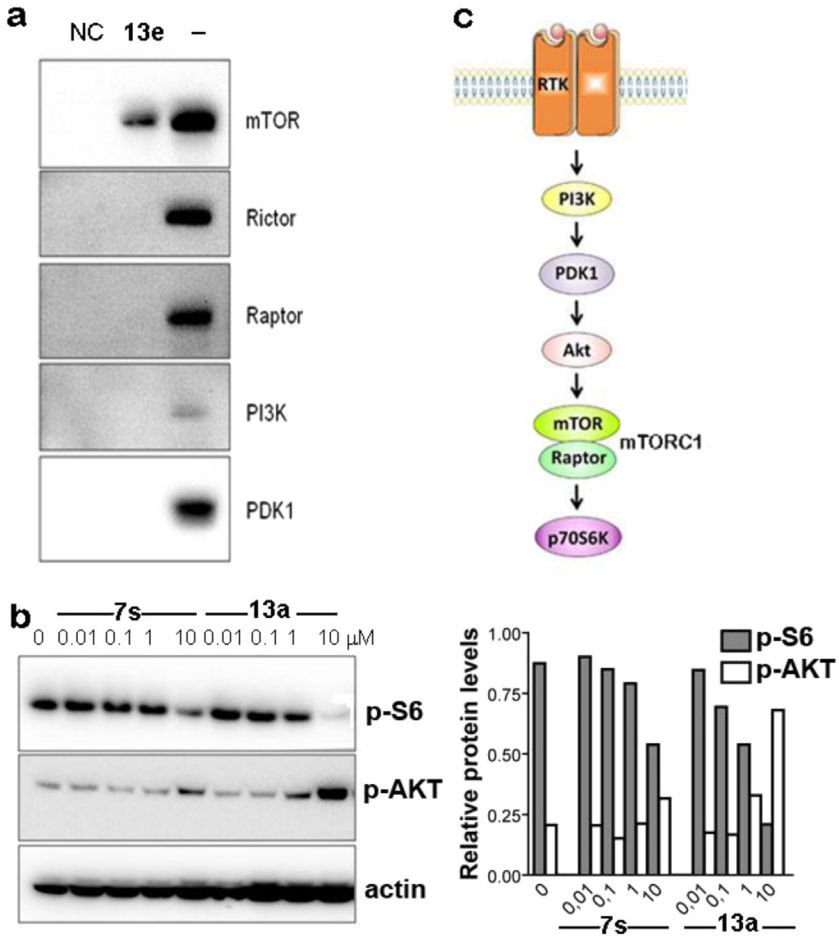


Fig. 3. Binding and inhibition of mTORC1. (a) Pull-down assay results demonstrating an interaction with mTOR or with a protein that interacts with mTOR. Western blot analysis of proteins pulled down from the negative control-coupled Affi-Gel beads (NC), **13d**-coupled Affi-Gel beads (**13e**) and the crude protein extract (–). (b) Inhibition of the phosphorylation of pS6 (mTORC1 substrate) and activation of p-AKT (Ser473) in SQ20B cells, exposed to **7s** or **13a** (0–10 μM) determined by immunoblotting of whole-cell lysates (left panel). Right panel: quantification of pS6 and pAKT expression relative to actin levels (c) Akt/mTOR signaling.

flash column chromatography on silica gel or by recrystallization in EtOH to obtain a white solid.

*4.2.2. 2-Benzyl-4-((dimethylamino)methyl)benzofuran-5-ol (**1**)*

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and dimethylamine (40 mg, 0.90 mmol), **1** (92 mg, 72%) was obtained after recrystallization in EtOH as a white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 7.21 (d, $J = 9.1$ Hz, 1H), 6.75 (d, $J = 9.1$ Hz, 1H), 6.24 (s, 1H), 4.08 (s, 2H), 3.76 (s, 2H), 2.35 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.4, 153.8, 149.0, 137.4, 129.1, 128.8, 128.1, 126.9, 112.7, 111.7, 110.4, 101.2, 59.5, 44.8, 35.3; IR (neat): 3029, 2995, 2953, 2915, 2850, 1611, 1494, 1450, 1423, 1228, 997, 972, 823, 737, 694; LC–MS ($M + H^+$)(ESI$^+$) 282.10 [$M + H^+$] (calcd for $C_{18}H_{19}NO_2H^+$ 282.10).

*4.2.3. (5-Methoxybenzofuran-2-yl)(phenyl)methanone (**4**)*

2-Hydroxy-5-methoxybenzaldehyde (**5**) (10 g, 65.7 mmol) was added to a 2-butanone solution (400 mL) of α-chloroacetophenone **6** (10.16 g, 65.7 mmol) and K_2CO_3 (10.9 g, 78.8 mmol). The mixture was stirred at reflux (5 h). Then, the volatiles were evaporated and the residue was dissolved in EtOAc. The organic layer was washed with a 2 N aqueous NaOH solution (2 × 50 mL), brine (2 × 50 mL), dried ($MgSO_4$) and concentrated under vacuum. The brown solid was recrystallized in EtOH to obtain brown crystals (16.20 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, $J = 8.4$ Hz, 2H), 7.61–7.45 (m, 5H), 7.09 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 153.0, 137.3, 132.8, 129.5, 128.5, 127.5, 118.5, 116.4, 113.2, 103.9, 55.9; IR (neat): 3119, 3058 (C–H ar), 2960, 2929, 2833 (C–H), 1644, 1598, 1576, 1547, 1470, 1445, 1224 (C–O methoxy), 1161, 970, 898, 831, 729, 697 cm^{-1}.

*4.2.4. 2-Benzyl-5-methoxybenzofuran (**5**)*

A solution of **4** (7.63 g, 30.3 mmol) and TMSCl (23 mL, 181.5 mmol) in anhydrous CH_3CN (100 mL) was cooled to 0 °C and $NaBH_3CN$ (11.41 g, 181.5 mmol) was added in one time. The mixture was stirred at room temperature (60 h). The solution was filtered through a pad of celite and the pad was washed with CH_2Cl_2 (200 mL). The organic layer was washed with a 2 N aqueous HCl solution (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using heptane/CH_2Cl_2 (1/1) as a solvent. Benzofuran **5** was obtained as yellow oil (6.49 g, 90%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.22–7.16 (m, 6H), 6.87–6.70 (m, 2H), 6.23 (s, 1H), 4.0 (s, 2H), 3.73 (s, 3H).

*4.2.5. 2-Benzylbenzofuran-5-ol (**6**)*

A 1 M CH_2Cl_2 solution of BBr_3 (35.9 mL, 35.9 mmol) was added, at −78 °C, to an anhydrous CH_2Cl_2 (100 mL) mixture of **5** (3.43 g, 14.4 mmol). The medium was stirred at room temperature (16 h). H_2O (20 mL) was added and the layers were separated. The organic

Table 2
Cytotoxicity of **7r**, **13a**, everolimus and OZI027 against human cancer cell lines (IC_{50}, μM).[a]

Cell lines	**7r**	**13a**	Everolimus	OZI027
Colo205 (colon)	3.1 ± 0.5	6.3 ± 2.2	20 ± 4.7	5.8 ± 0.6
ColoR (colon)	3.7 ± 0.5	18 ± 1.5	8.7 ± 1.9	5.8 ± 1.4
HCT116 (colon)	12 ± 1.9	57 ± 4.2	12 ± 5.1	5.6 ± 1.0
HT29 (colon)	5.1 ± 1.0	45 ± 5.9	15 ± 2.8	55 ± 4.2
CAKI1 (kidney)	6.9 ± 1.7	26 ± 2.0	14 ± 2.5	1.9 ± 0.3
SK-HEP1 (liver)	11 ± 2.1	42 ± 7.7	12 ± 1.8	3.2 ± 1.3
DU145 (prostate)	20 ± 1.6	13 ± 1.8	8.0 ± 2.1	4.1 ± 0.8
OVCAR3 (ovary)	19 ± 3.3	22 ± 3.4	16 ± 2.6	8.1 ± 3.3
HOP62 (lung)	11 ± 3.4	54 ± 6.1	19 ± 3.3	41 ± 7.2
SQ20B (head and neck)	3.0 ± 0.9	2.8 ± 0.7	5.5 ± 1.6	1.3 ± 0.4

[a] Data are the average of two independent IC_{50} value determinations.

layer was washed with H_2O (20 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 20 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using heptane/EtOAc (8/2) as a solvent to obtain **6** [11] as a yellow oil (2.70 g, 84%): *Rf* 0.22 (heptane/EtOAc; 8/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.16 (m, 6H), 6.81 (d, *J* = 2.6 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 6.20 (s, 1H), 4.58 (s, 1H), 4.0 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 151.3, 150.1, 137.2, 129.7, 128.9, 128.6, 126.8, 111.8, 111.2, 105.6, 103.3, 35.1; IR (neat): 3166 (OH phenol), 2920, 2853, 1621, 1599, 1494, 1469, 1194, 1170, 950, 899, 837, 741, 707 cm^{-1}; LC–MS (M + H^+)(ESI^+) 225.10 [M + H^+] (calcd for $C_{15}H_{12}O_2H^+$ 225.10).

4.2.6. 2-Benzyl-4-((diethylamino)methyl)benzofuran-5-ol (*7a*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and diethylamine (66 mg, 0.90 mmol), **7a** (90 mg, 65%) was obtained as an oil after purification by column chromatography on silica gel using pentane/Et_2O (8/2) as solvent: *Rf* (pentane/Et_2O; 1/1) 0.43; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27–7.17 (m, 5H), 7.09 (d, *J* = 8.6 Hz, 1H), 6.62 (d, *J* = 8.6 Hz, 1H), 6.13 (s, 1H), 3.98 (s, 2H), 3.78 (s, 2H), 2.55 (q, *J* = 7.1 Hz, 4H), 1.03 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 153.8, 148.8, 137.3, 128.9, 128.6, 126.7, 112.6, 111.7, 110.0, 101.0, 53.5, 46.7, 35.2, 11.4; IR (neat): 2967, 2925, 2847 (C–H), 1612, 1494 (C=C ar), 1443 (C–H CH_3), 1229 (C–O phenol), 1195, 1135, 958, 792, 771; 738, 702; LC–MS (M + H^+)(ESI^+) 310.1 [M + H^+] (calcd for $C_{20}H_{23}NO_2H^+$ 310.2).

4.2.7. 2-Benzyl-4-((cyclohexyl(methyl)amino)methyl)benzofuran-5-ol (*7b*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-methyl-cyclohexylamine (102 mg, 0.90 mmol), **7b** (38 mg, 24%) was obtained as an oil after purification by column chromatography on silica gel using pentane/EtOAc (9/1) as eluant: *Rf* (Pentane/EtOAc; 8/2) = 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.27 (m, 5H), 7.19 (d, *J* = 8.9 Hz, 1H), 6.72 (d, *J* = 8.9 Hz, 1H), 6.23 (s, 1H), 4.08 (s, 2H), 3.93 (s, 2H), 2.6 (m, 1H), 2.3 (s, 3H), 1.93–0.91 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.9, 153.7, 148.5, 137.0, 128.7, 128.4, 127.7, 126.5, 112.3, 111.4, 109.7, 100.8, 62.1, 53.7, 36.4, 34.9, 27.9, 25.8, 25.5; IR (neat): 2926 (C–H); 2852 (C–H CH_3); 1612, 1494 (C=C ar), 1449 (C–H CH_2), 1230 (C–O phenol), 796; 734; 702 (C–H); LC–MS (M + H^+)(ESI^+) 350.2 [M + H^+] (calcd for $C_{23}H_{27}NO_2H^+$ 350.2).

4.2.8. 2-Benzyl-4-((3,4-dihydroisoquinolin-2(1H)-yl)methyl) benzofuran-5-ol (*7c*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and tetrahydroisoquinoline (120 mg, 0.90 mmol), **7c** (92 mg, 55%) was obtained after recrystallization in EtOH/EtOAc as white crystals: *Rf* (pentane/Et_2O; 8/2) 0.36; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39–7.11 (m, 10H), 7.02 (d, *J* = 7.8 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 1H), 6.28 (s, 1H), 4.09 (s, 2H), 4.0 (s, 2H), 3.79 (s, 2H), 2.98 (t, *J* = 5.3 Hz, 2H), 2.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 153.5, 149.0, 137.2, 133.6, 133.4, 129.0, 128.7, 128.6, 128.4, 128.3, 126.8, 126.6, 125.9, 112.7, 110.8, 110.4, 101.1, 57.6, 55.6, 50.2, 35.2, 28.7; IR (neat): 3027 (C–H ar), 2799 (C–H); 1601, 1583, 1494 (C=C ar), 1249 (C–O phenol), 1226, 793; 740, 701; LC–MS (M + H^+)(ESI^+) 370.1 [M + H^+] (calcd for $C_{25}H_{23}NO_2H^+$ 370.2).

4.2.9. 2-Benzyl-4-((benzyl(methyl)amino)methyl)benzofuran-5-ol (*7d*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-methyl-benzylamine (109 mg, 0.90 mmol), **7d** (140 mg, 44%) was obtained as an oil after purification by column chromatography on silica gel using pentane/*t*-BuOMe (9/1) as solvent: *Rf* (pentane/Et_2O; 1/1) 0.77; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.15 (m, 10H), 7.12 (d, *J* = 10.7 Hz, 1H), 6.67 (d, *J* = 8.0 Hz, 1H), 6.15 (s, 1H), 3.99 (s, 2H), 3.76 (s, 2H), 3.54 (s, 2H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 153.4, 148.9, 137.2, 136.8, 129.4, 128.9, 128.6, 128.5, 128.1, 127.6, 126.8, 112.6, 111.5, 110.3, 101.1, 61.5, 57.2, 41.5, 35.2; IR (neat): 3027 (C–H ar), 2838 (C–H), 1611, 1493 (C=C ar), 1445 (C–H CH_3), 1229 (C–O phenol), 1029, 994, 957, 853, 792, 731, 698; LC–MS (M + H^+)(ESI^+) 358.1 [M + H^+] (calcd for $C_{24}H_{23}NO_2H^+$ 358.2).

4.2.10. 2-Benzyl-4-((methoxy(methyl)amino)methyl)benzofuran-5-ol (*7e*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N,O*-dimethylhydroxylamine (55 mg, 0.90 mmol), **7e** (68 mg, 50%) was obtained as an oil after purification by column chromatography on silica gel using Pentane/EtOAc (9/1) as solvent: *Rf* (pentane/EtOAc; 9/1) 0.33; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.37–7.25 (m, 6H), 6.81 (d, *J* = 8.8 Hz, 1H), 6.31 (s, 1H), 4.2–3.8 (br s, 3H), 4.10 (s, 2H), 3.59 (s, 2H), 2.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 152.3, 149.2, 137.2, 128.9, 128.6, 126.8, 112.8, 111.5, 110.7, 101.3, 60.2, 59.4, 44.2, 35.2; IR (neat): 3028 (C–H ar), 2892 (C–H); 1603, 1494, 1439 (C–H CH_3), 1230 (C–O phenol), 1074, 865, 789; 735, 702; LC–MS (M–NH(CH_3)(OCH_3))(ESI^+) 237.1 [M – NH(CH_3)(OCH_3)]$^+$ (calcd for $C_{16}H_{13}O_2^+$ 237.1).

4.2.11. 2-Benzyl-4-(((2-hydroxyethyl)(methyl)amino)methyl) benzofuran-5-ol (*7f*)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-methyl-2-aminoethanol (68 mg, 0.90 mmol), **7f** (36 mg, 27%) was obtained as an oil after purification by column chromatography on silica gel using EtOAc as solvent: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (m, 5H), 7.21 (d, *J* = 8.7 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.23 (s, 1H), 5.52–5.01 (br m, 1H), 4.07 (s, 2H), 3.87 (s, 2H), 3.8 (t, *J* = 5.5 Hz, 2H), 2.7 (t, *J* = 5.5 Hz, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 153.4, 149.1, 137.3, 129.1, 128.7, 128.2, 126.9, 112.8, 111.7, 110.5, 101.2, 59.9, 59.1, 58.0, 42.2, 35.3; IR (neat): 3380 (OH); 2980 (C–H); 1610, 1494 (C=C ar); 1436 (C–H), 1420; 1228 (C–O phenol); 792; 734, 702; LC–MS (M + H^+)(ESI^+) 312.2 [M + H^+] (calcd for $C_{19}H_{21}NO_3H^+$ 312.1).

4.2.12. 2-Benzyl-4-((cyclohexyl(2-hydroxyethyl)amino)methyl) benzofuran-5-ol (*7g*)

In a sealed tube, **6** (200 mg, 0.90 mmol), 2-(cyclohexylamino) ethanol (390 μL, 4.5 mmol) and formaldehyde (37% in water, 363 μL, 4.5 mmol) were stirred at room temperature (6 d) in dioxane (2 mL). The volatiles were evaporated and the crude residue was

purified by flash chromatography on silica gel using pentane/Et_2O (1/1) as solvent. Concentrated HCl (2 mL, 23.5 mmol) was added to a BuOH/H_2O (10/1) solution of the purified residue. The solution was stirred at reflux (4 h). The mixture was concentrated under vacuum and the aqueous layer was basified with a saturated Na_2CO_3 aqueous solution and washed with Et_2O (3 × 30 mL). The organic layers were combined dried ($MgSO_4$), and concentrated under vacuum. The crude residue was purified by flash chromatography on silica gel using CH_2Cl_2/MeOH/Et_3N (9/1/0.1) as solvent to obtain 56 mg of **7g** (32%); ^{1}H NMR (400 Hz, $CDCl_3$) δ 7.36–7.14 (m, 5H), 7.17 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.7 Hz, 1H), 6.23 (s, 1H), 4.08 (s, 2H), 4.00 (s, 2H), 3.74–3.69 (m, 3H), 2.78–2.70 (m, 3H), 1.94–1.80 (m, 2H), 1.36–1.15 (m, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7, 153.9, 149.4, 137.6, 129.4, 129.0, 128.3, 127.2, 113.2, 112.4, 110.5, 101.4, 61.1, 60.7, 53.2, 51.6, 35.6, 28.4, 26.2, 26.0; IR (neat): 3028 (C–H ar), 2921 (CH_2), 1603, 1494 (C=C ar), 1434 (C–H CH_3), 1373 (N–H amine), 1232 (C–O phenol), 1174, 953, 791,732, 699; LC–MS (M + H^+)(ESI^+) 268.1 [M + H^+] (calcd for $C_{17}H_{17}NO_2H^+$ 268.1).

4.2.13. 2,2′-(((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl) azanediyl)diethanol (**7h**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and diethanolamine (95 mg, 0.90 mmol), **7h** (32 mg, 21%) was obtained as a colorless oil after purification by column chromatography on silica gel using EtOAc/MeOH (9/1): *Rf* (EtOAc/MeOH; 9/1) 0.3; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.25 (m, 5H), 7.18 (d, J = 8.7 Hz, 1H), 6.73 (d, J = 8.7 Hz, 1H), 6.24 (s, 1H), 5.55–5.15 (br m, 3H), 4.14 (s, 2H), 3.96 (s, 2H), 3.75 (t, J = 5.1 Hz, 4H), 2.77 (t, J = 5.1 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 153.2, 149.4, 137.5, 129.3, 129.0, 128.7, 127.2, 113.2, 112.5, 110.8, 101.4, 60.5, 56.7, 56.0, 35.5; IR (neat): 3270 (OH), 2917, 2849 (C–H), 1602, 1574, 1567, 1556 (C=C ar), 1434 (C–H CH_2), 1227 (C–O phenol), 1070 (OH), 956, 792; 702; LC–MS (M + H^+)(ESI^+) 342.1 [M + H^+] (calcd for $C_{20}H_{23}NO_4H^+$ 342.2).

4.2.14. 2-Benzyl-4-(morpholinomethyl)benzofuran-5-ol (**7i**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and morpholine (91 mg, 0.90 mmol), **7i** (74 mg, 51%) was obtained after recrystallization in EtOH as a white solid: *Rf* (pentane/EtOAc; 8/2) = 0.73; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.5 (br s, 1H), 7.39–7.28 (m, 5H), 7.23 (d, J = 8.8 Hz, 1H), 6.75 (d, J = 8.8 Hz, 1H), 6.26 (s, 1H), 4.09 (s, 2H), 3.84 (s, 2H), 3.78 (t, J = 4.0 Hz, 4H), 2.61 (br s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.5, 153.1, 149.0, 137.1, 128.9, 128.6, 128.3, 126.8, 112.6, 110.5, 110.3, 101.0, 66.8, 58.3, 53.1, 35.2; IR (neat): 2970, 2852 (C–H), 1600, 1494 (C=C ar), 1454 (C–H CH_2), 1227 (C–O phenol), 1113, 858, 794, 783; 735, 700; LC–MS (M + H^+)(ESI^+) 324.10 [M + H^+] (calcd for $C_{20}H_{21}NO_3H^+$ 324.10).

4.2.15. 2-Benzyl-4-((4-methylpiperazin-1-yl)methyl)benzofuran-5-ol (**7j**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-methylpiperazine (90 mg, 0.90 mmol), **7j** (145 mg, 95%) was obtained as a yellow solid after purification by column chromatography on silica gel using EtOAc/MeOH (9/1) as solvent: *Rf* (EtOAc/MeOH; 9/1) 0.32; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.18 (m, 6H), 6.72 (d, J = 8.7 Hz, 1H), 6.25 (s, 1H), 4.07 (s, 2H), 3.83 (s, 2H), 2.69–2.38 (br m, 8H), 2.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7, 153.6, 149.3, 138.9, 129.3, 129.0, 128.6, 127.1, 112.9, 111.1, 110.7, 101.5, 58.2, 55.3, 53.0, 46.2, 35.5; IR (neat): 2921, 2849 (C–H), 2801; 1607, 1488 (C=C ar), 1440 (C–H CH_3), 1223 (C–O phenol), 1144, 1132, 806; 738, 700; LC–MS (M + H^+)(ESI^+) 337.2 [M + H^+] (calcd for $C_{21}H_{24}N_2O_2H^+$ 337.2).

4.2.16. 2-Benzyl-4-((4-benzylpiperazin-1-yl)methyl)benzofuran-5-ol (**7k**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-benzyl-piperazine (158 mg, 0.90 mmol), **7k** (72 mg, 39%) was obtained after recrystallization in EtOH as white crystals: *Rf* (EtOAc/heptane; 1/1) 0.38; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.21 (m, 10H), 7.20 (d, J = 8.8 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 6.24 (s, 1H), 4.08 (s, 2H), 3.83 (s, 2H), 3.56 (s, 2H), 2.61–2.58 (br m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 153.3, 148.9, 137.2, 129.1, 128.9, 128.6, 128.3, 128.2, 127.2, 127.2, 126.7, 112.5, 110.7, 110.3, 101.3, 62.8, 57.8, 52.8, 52.7, 35.2; IR (neat): 3029 (C–H ar); 2810 (C–H); 1600, 1494, 1443 (C=C ar); 1227 (C–O phenol); 1130, 952, 795, 783; 735, 698 (C–H); LC–MS (M + H^+)(ESI^+) 413.2 [M + H^+] (calcd for $C_{27}H_{28}N_2O_2H^+$ 413.2).

4.2.17. 2–Benzyl-4-((4-(2-(2-hydroxyethoxy)ethyl)piperazin-1-yl) methyl)benzofuran-5-ol (**7l**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 1-[2-(2-hydroxyethoxy) ethyl]piperazine (157 mg, 0.90 mmol), **7l** (90 mg, 65%) was obtained as an oil after purification by column chromatography on silica gel using EtOAc/MeOH (8/2) as solvent; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.19 (m, 6H), 6.72 (d, J = 8.8 Hz, 1H), 6.23 (s, 1H), 4.08 (s, 2H), 3.84 (s, 2H), 3.74–3.62 (m, 6H), 2.89–2.06 (br m, 10H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 153.3, 149.1, 137.3, 129.1, 128.8, 128.5, 127.0, 112.7, 110.9, 110.5, 101.3, 72.6, 67.6, 62.2, 57.9, 5, 52.53, 35.3.

4.2.18. 4-((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl)piperazine-1-carbaldehyde (**7m**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-formyl-piperazine (103 mg, 0.90 mmol), **7m** (135 mg, 50%) was obtained as a white solid after purification by column chromatography on silica gel using pentane/EtOAc (1/1) as solvent: *Rf* (pentane/EtOAc; 1/1) 0.17; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.28–7.13 (m, 6H), 6.65 (d, J = 8.8 Hz, 1H), 6.13 (s, 1H), 3.98 (s, 2H), 3.76 (s, 2H), 3.55 (m, 2H), 3.36 (t, J = 5.0 Hz, 2H), 2.49 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.6, 158.7, 152.9, 149.9, 137.1, 128.9, 128.6, 128.4, 126.8, 112.6, 110.7, 110.1, 100.9, 57.8, 53.0, 45.3, 39.7, 35.2; IR (neat): 2960, 2845 (C–H), 1698, 1682 (C=O amide), 1651 (N–H amide), 1600, 1494 (C=C ar), 1434 (C–H CH_2), 1269, 1227 (C–O phenol), 1133, 1022, 793, 782; 734, 700; LC–MS (M + H^+)(ESI^+) 351.1 [M + H^+] (calcd for $C_{21}H_{22}N_2O_3H^+$ 351.2).

4.2.19. 1-((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl)piperidin-3-ol (**7n**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 3-hydroxypiperidine (91 mg, 0.90 mmol), **7n** (43 mg, 28%) was obtained as an oil after purification by column chromatography on silica gel using EtOAc as solvent: *Rf* (EtOAc) 0.51; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.20 (m, 6H), 6.74 (d, J = 8.8 Hz, 1H), 6.24 (s, 1H), 4.09 (s, 2H), 3.828–3.80 (s, 3H), 2.29–1.27 (m, 9H); RMN ^{13}C (100 MHz, $CDCl_3$) δ 158.4, 153.2, 149.0, 137.2, 128.9, 128.6, 128.2, 126.8, 112.6, 110.9, 110.3, 101.1, 66.8, 60.1, 58.0, 53.1, 35.2, 32.4, 22.7; IR (neat): 3386 (OH), 3027 (C–H ar), 2938, 2808 (C–H), 1610, 1494 (C=C ar), 1441 (C–H CH_2), 1347 (OH), 1228 (C–O phenol), 1153, 1107 (OH), 959, 791; 730, 702; LC–MS (M + H^+)(ESI^+) 338.1 [M + H^+] (calcd for $C_{21}H_{23}NO_3H^+$ 338.2).

4.2.20. 1-((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl)piperidin-4-ol (**7o**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-hydroxypiperidine (91 mg, 0.90 mmol), **7o** (28 mg, 18%) was obtained after

recrystallization in EtOH as white crystals: *Rf* (pentane/Et_2O, 1/1) 0.09; ^{1}H NMR (400 Hz, $CDCl_3$) δ 7.38–7.20 (m, 6H), 6.74 (d, J = 8.8 Hz, 1H), 6.23 (s, 1H), 4.08 (s, 2H), 3.82 (s, 2H), 2.95–2.80 (br s, 2H), 2.31–2.45 (br s, 2H), 1.99–1.94 (m, 2H), 1.72–1.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 158.3, 153.4, 148.9, 137.2, 128.9, 128.6, 128.1, 126.7, 112.6, 111.0, 110.2, 101.0, 57.8, 35.2, 34.1; IR (neat): 3306 (OH secondary alcohol), 2944, 2825 (C–H), 1610, 1504, 1494 (C=C ar), 1447 (C–H CH_2), 1054; 1228 (C–O phenol), 1054, 1016, 799, 793, 779; 735, 699; LC–MS (M + H^+)(ESI^+) 338.1 [M + H^+] (calcd for $C_{21}H_{23}NO_3H^+$ 338.2).

4.2.21. 1-((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl)piperidine-4-carboxamide (**7p**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-carboxamidepiperidine (115 mg, 0.90 mmol), **7p** (28 mg, 17%) was obtained after recrystallization in EtOH as white crystals: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.11 (m, 6H), 6.64 (d, J = 8.8 Hz, 1H), 6.15 (s, 1H), 4.0 (s, 2H), 3.74 (s, 2H), 3.01 (d, J = 11.6 Hz, 2H), 2.20–2.10 (br m, 4H), 1.95–1.68 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 152.9, 148.9, 137.1, 128.9, 128.6, 128.2, 126.7, 112.4, 110.8, 110.2, 101.0, 57.9, 52.6, 39.1, 35.1, 28.6; IR (neat): 3331, 3165 (N–H amide), 2928, 2812 (C–H), 1614 (C=O amide), 1494 (C=C ar), 1443, 1434 (C–H CH_2), 1416, 1229 (C–O phenol), 1142, 800, 792, 779; 734, 699; LC–MS (M + H^+)(ESI^+) 365.1 [M + H^+] (calcd for $C_{22}H_{24}N_2O_3H^+$ 364.2).

4.2.22. 2-Benzyl-4-((4-phenylpiperidin-1-yl)methyl)benzofuran-5-ol (**7q**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-phenylpiperidine (145 mg, 0.90 mmol), **7q** (108 mg, 61%) was obtained after recrystallization in EtOH as white crystals: *Rf* (pentane/EtOAc; 9/1) 0.32; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.23 (m, 11H), 6.79 (d, J = 8.8 Hz, 1H), 6.29 (s, 1H), 4.12 (s, 2H), 3.89 (s, 2H), 3.20 (d, J = 11.6 Hz, 2H), 2.64 (m, 1H), 2.36–2.25 (td, J = 3.2, 11.6 Hz, 2H), 1.96–1.86 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 153.6, 148.9, 145.6, 137.3, 129.0, 128.7, 128.5, 128.2, 126.9, 126.8, 126.4, 112.6, 111.2, 110.2, 101.1, 58.3, 53.9, 42.4, 35.2, 33.4; IR (neat): 3028 (C–H ar), 2928, 2808 (C–H), 1603, 1493 (C=C ar), 1451 (C–H CH_2), 1230 (C–O phenol), 1142; 731, 700; LC–MS (M + H^+)(ESI^+) 398.2 [M + H^+] (calcd for $C_{27}H_{27}NO_2H^+$ 398.2).

4.2.23. 4-((1,4′-Bipiperidin)-1′-ylmethyl)-2-benzylbenzofuran-5-ol (**7r**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-piperidopiperidine (151 mg, 0.90 mmol), **7r** (74 mg, 41%) was obtained after recrystallization in MeOH as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12–7.04 (m, 5H), 6.96 (d, J = 8.8 Hz, 1H), 6.49 (d, J = 8.8 Hz, 1H), 5.99 (s, 1H), 3.84 (s, 2H), 3.56 (s, 2H), 2.86 (d, J = 10.4 Hz, 2H), 2.27–1.21 (m, 17H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 153.4, 148.9, 137.2, 128.9, 128.6, 128.1, 126.7, 112.6, 111.2, 110.1, 101.1, 62.3, 57.9, 53.1, 50.2, 35.2, 27.9, 26.4, 24.8; IR (neat): 2958, 2933, 2848 (C–H), 2793, 2756; 1613, 1598, 1493 (C=C ar); 1446 (C–H CH_2), 1228 (C–O phenol), 1101, 953, 792, 782; 735, 699; LC–MS (M + H^+)(ESI^+) 405.3 [M + H^+] (calcd for $C_{26}H_{32}N_2O_2H^+$ 405.2).

4.2.24. 2-Benzyl-4-((4-(pyrrolidin-1-yl)piperidin-1-yl)methyl) benzofuran-5-ol (**7s**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-(pyrrolidin-1-yl)piperidine (139 mg, 0.90 mmol), **7s** (116 mg, 66%) was obtained as a white solid after filtration; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.44 (m, 5H), 7.15 (d, J = 8.6 Hz, 1H), 6.69 (d, J = 8.6 Hz, 1H), 6.19 (s, 1H), 4.04 (s, 2H), 3.76 (s, 2H), 2.99 (d, J = 11.6 Hz, 2H), 2.55–2.50 (m, 4H), 2.16–2.03 (m, 3H), 1.91 (d, J = 12.8 Hz, 2H), 1.78–1.60 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 153.5, 148.9, 137.3, 129.0, 128.6, 128.1, 126.8, 112.7, 111.1, 110.1, 101.1, 61.3, 57.9, 52.0, 51.4, 35.2, 31.3, 23.3.

4.2.25. 1′-((2-Benzyl-5-hydroxybenzofuran-4-yl)methyl)-N-(2-(2-hydroxyethoxy)ethyl)-[1,4′-bipiperidine]-4-carboxamide (**7t**)

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and *N*-(2-(2-hydroxyethoxy) ethyl)-[1,4′-bipiperidine]-4-carboxamide (269 mg, 0.90 mol), **7t** (90 mg, 65%) was obtained as an oil after purification by column chromatography on silica gel using acetone as solvent; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 7.6 Hz, 2H), 7.34–7.15 (m, 5H), 6.22 (s, 1H), 5.59 (br t, 1**H**), 4.08 (s, 2H), 3.81–3.76 (m, 5H), 3.59 (q, J = 4.8 Hz, 4H), 3.52–3.43 (m, 2H), 3.10 (d, J = 12.0 Hz, 2H), 2.99 (d, J = 11.6 Hz, 2H), 2.42–2.37 (m, 1H), 2.18–2.10 (m, 5H), 1.99–1.58 (m, 8H).

4.2.26. 2-Benzoylbenzofuran-5-ol (**8**)

A 1 M CH_2Cl_2 solution of BBr_3 (35.9 mL, 35.9 mmol) was added, at −78 °C, to an anhydrous CH_2Cl_2 (100 mL) mixture of **4** (3.43 g, 14.4 mmol). The medium was stirred at room temperature (16 h). H_2O (20 mL) was added and the layers were separated. The organic layer was washed with H_2O (20 mL). The aqueous layers were combined and washed with CH_2Cl_2 (2 × 20 mL). The organic layers were combined, dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using heptane/EtOAc (8/2) as a solvent to obtain **8** as a yellow oil (2.70 g, 84%): *Rf* 0.22 (heptane/EtOAc; 8/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.0 Hz, 2H), 7.54–7.32 (m, 5H), 6.93–6.98 (m, 2H), 3.66 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.0, 153.7, 152.6, 151.0, 137.2, 133.0, 129.4, 128.6, 127.8, 118.6, 117.1, 112.9, 106.8.

4.2.27. (4-((1,4′-Bipiperidin)-1′-ylmethyl)-5-hydroxybenzofuran-2-yl)(phenyl)methanone (**10**)

Using Method A with formaldehyde (224 μL, 2.52 mmol), **8** [12] (300 mg, 1.26 mmol) and 4-piperidinopiperidine (424 mg, 2.52 mmol), **10** (500 mg, 95%) was obtained as a white solid after purification by column chromatography on silica gel using EtOAc/MeOH (7/3) as solvent; *Rf* = 0.19 (EtOAc/MeOH 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07–8.04 (m, 2H), 7.66–7.62 (m, 1H), 7.57–7.52 (m, 2H), 7.44 (s, 1H), 7.42 (d, J = 8.8 Hz, 1H), 7.03 (d, J = 8.8 Hz, 1H), 3.91 (s, 2H), 3.03–2.98 (m, 2H), 2.53–2.50 (m, 4H), 2.38–2.31 (m, 1H), 2.20–2.14 (m, 2H), 1.88 (d, J = 12.8 Hz, 2H), 1.71–1.54 (m, 6H), 1.48–1.43 (m, 2H); ^{13}C NMR (100 MHz $CDCl_3$) δ 184.1, 154.5, 152.8, 150.4, 137.3, 132.8, 129.5, 128.5, 126.5, 118.9, 113.9, 112.7, 111.9, 62.1, 57.7, 53.2, 50.3, 27.9, 26.4, 24.7.

4.2.28. 4-((1,4′-Bipiperidin)-1′-ylmethyl)-2-(hydroxy(phenyl) methyl)benzofuran-5-ol (**11**)

$NaBH_4$ (27 mg, 0.72 mmol) was added at 0 °C to a MeOH (8 mL) solution of the benzofuran **10** (100 mg, 0.24 mmol). The mixture was stirred at room temperature (3 h). Water was added cautiously. The white precipitate was filtered and recrystalized in MeOH to obtain 92 mg (90%) of **11** as white crystals; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.45 (m, 2H), 7.39–7.28 (m, 3H), 7.17 (d, J = 8.8 Hz, 1H), 6.72 (d, J = 8.8 Hz, 1H), 6.34 (s, 1H), 5.85 (s, 1H), 3.72 (s, 2H), 3.03–2.98 (m, 2H), 2.47–2.42 (m, 4H), 2.27–2.20 (m, 1H), 2.05–1.97 (m, 2H), 1.80–1.75 (m, 2H), 1.62–1.54 (m, 6H), 1.42–1.40 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 153.6, 149.1, 140.7, 128.6, 128.2, 127.4, 126.8, 113.6, 111.7, 110.6, 101.6, 70.5, 62.2, 57.8, 53.1, 50.2, 27.7, 26.2, 24.7.

*4.2.29. 4-((1,4′-Bipiperidin)-1′-ylmethyl)-2-phenylbenzofuran-5-ol (**13a**)*

Using Method A with formaldehyde (68 μL, 0.90 mmol), benzofuran **6** (100 mg, 0.45 mmol) and 4-piperidinopiperidine (151 mg, 0.90 mmol), **13a** (90 mg, 65%) was obtained as an oil after purification by column chromatography on silica gel using acetone as solvent; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 7.6 Hz, 2H), 7.34–7.30 (m, 2H), 7.25–7.14 (m, 2H), 6.80 (s, 1H), 6.68 (d, J = 8.7 Hz, 1H), 3.78 (s, 2H), 3.03 (d, J = 12.0 Hz, 2H), 2.42–2.39 (m, 4H), 2.27–2.21 (m, 1H), 2.08–2.018 (m, 2H), 1.76 (d, J = 12.8 Hz, 2H), 1.61–1.46 (m, 6H), 1.36–1.34 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 153.8, 149.0, 130.6, 128.8, 128.6, 128.4, 124.8, 113.7, 111.4, 110.5, 99.1, 62.3, 57.9, 53.2, 50.3, 27.9, 26.4, 24.8.

*4.2.30. N-(20-Azido-3,6,9,12,15,18-hexaoxaicosyl)-1′-((5-hydroxy-2-phenylbenzofuran-4-yl)methyl)-(1,4′-bipiperidine)-4-carboxamide (**13c**)*

Using Method A with formaldehyde (70 μL, 0.840 mmol), benzofuran **12** (94 mg, 0.42 mmol) and ethyl (1,4′-bipiperidine)-4-carboxylate [13] (151 mg, 0.90 mol), **13b** (161 mg, 80%) was obtained as an oil after purification by column chromatography on silica gel using acetone as solvent. EDCI (25 mg, 0.129 mmol), HOBT (17 mg, 0.129 mmol) were added to a CH_2Cl_2 (4 mL) solution of **13b** (54 mg, 0.108 mmol). The solution was stirred at 0 °C (15 min). A CH_2Cl_2 (2 mL) solution of 20-azido-3,6,9,12,15,18-hexaoxaicosan-1-amine (38 mg, 0.108 mmol) and DIEA (36 μL, 0.280 mmol) was added dropwise and the solution was stirred at room temperature (16 h). H_2O (5 mL) was added and the layers were separated. The organic layer was washed with EtOAc (3 × 5 mL). The organic layers were combined, dried and concentrated under vacuum. The crude residue was purified by chromatography on silica gel using MeOH as eluent to obtain 7 mg (8%) of **13c** as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 7.3 Hz, 2H), 7.49–7.40 (m, 2H), 7.38–7.30 (m, 2H), 6.92 (s, 1H), 6.80 (d, J = 8.8 Hz, 1H), 6.28 (s, 1H), 3.92 (s, 2H), 3.72–3.61 (m, 22H), 3.56 (t, J = 5.0 Hz, 2H), 3.48 (app q, J = 5.0 Hz, 2H), 3.40 (t, J = 5.3 Hz, 2H), 3.15 (d, J = 11.2 Hz, 2H), 3.02 (d, J = 11.2 Hz, 2H), 2.52–2.09 (m, 6H), 1.94–1.68 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 156.3, 153.7, 149.0, 130.6, 128.8, 128.6, 128.5, 124.8, 113.3, 111.3, 110.5, 99.1, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.3, 70.2, 70.1, 70.0, 61.8, 57.8, 52.9, 50.7, 48.8, 39.1, 29.7, 28.9, 27.8; LC–MS (M + H$^+$)(ESI$^+$) 767.4 [M + H$^+$] (calcd for $C_{40}H_{58}N_6O_9H^+$ 767.4).

*4.2.31. N-(20-Amino-3,6,9,12,15,18-hexaoxaicosyl)-1′-((5-hydroxy-2-phenylbenzofuran-4-yl)methyl)-(1,4′-bipiperidine)-4-carboxamide (**13d**)*

Pd/C (5%, 1 mg) was added to an EtOH (5 mL) solution of **13c** (5 mg, 0.01 mmol). The mixture was stirred at room temperature and under 40 psi of hydrogen (16 h). The mixture was filtered on a pad of celite. The pad was washed with EtOH and the filtrate was evaporated to obtain 5 mg (quant.) of **13d** as a colorless oil which was conjugated to Affigel-10 without any further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 7.2 Hz, 2H), 7.48–7.42 (m, 2H), 7.38–7.30 (m, 2H), 6.93 (s, 1H), 6.80 (d, J = 8.8 Hz, 1H), 3.92 (s, 2H), 3.71–3.58 (m, 24H), 3.50–3.43 (app q, J = 5.0 Hz, 2H), 3.15 (d, J = 11.5 Hz, 2H), 3.08–2.95 (m, 4H), 2.26–2.14 (m, 6H), 1.93–1.70 (m, 8H); LC–MS (M + H$^+$)(ESI$^+$) 740.4 [M + H$^+$] (calcd for $C_{40}H_{60}N_4O_9H^+$ 741.4).

Acknowledgment

We are grateful to region Alsace and MNESR for fellowships to Christophe Salomé and Frédéric Thuaud. We thank also the *SATT Ile-de-France Innov* for financial support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.020.

References

[1] S. Albert, M. Serova, C. Dreyer, M.P. Sablin, S. Faivre, E. Raymond, New inhibitors of the mammalian target of rapamycin signaling pathway for cancer, Expert Opin. Investig. Drugs 19 (2010) 919–930.
[2] S. Faivre, G. Kroemer, E. Raymond, Current development of mTOR inhibitors as anticancer agents, Nat. Rev. Drug. Discov. 5 (2006) 671–688.
[3] S. Vignot, S. Faivre, D. Aguirre, E. Raymond, mTOR-targeted therapy of cancer with rapamycin derivatives, Ann. Oncol. 16 (2005) 525–537.
[4] T.R. Kau, F. Schroeder, S. Ramaswamy, C.L. Wojciechowski, J.J. Zhao, T.M. Roberts, J. Clardy, W.R. Sellers, P.A. Silver, A chemical genetic screen identifies inhibitors of regulated nuclear export of a Forkhead transcription factor in PTEN-deficient tumor cells, Cancer Cell. 4 (2003) 463–476.
[5] I. Smukste, B.R. Stockwell, Restoring functions of tumor suppressors with small molecules, Cancer Cell. 4 (2003) 419–420.
[6] J.F. Cheng, B.N. Nguyen, X. Liu, G.D. Lopaschuk, J.R. Dyck, Preparation of Heterocyclic Compounds Useful as Malonyl-CoA Decarboxylase Inhibitors, US 7696365 B2, 2005.
[7] V.G.S. Box, P.C. Meleties, Reductive, selective deoxygenation of acylbenzo[*b*]furans, aromatic aldehydes and ketones with $NaBH_3CN$-TMSCl, Tetrahedron Lett. 39 (1998) 7059–7062.
[8] V.M. Lyubchanskaya, V.S. Velezheva, I.S. Nikolaeva, E.A. Golovanova, L.M. Alekseeva, A.N. Fomina, 2-Benzyl(isobutyl)-5-hydroxybenzofuran derivatives: synthesis and antiviral activity, Khim.-Farm. Zh. 23 (1989) 843–847.
[9] K.E. O'Reilly, F. Rojo, Q.-B. She, D. Solit, G.B. Mills, D. Smith, H. Lane, F. Hofmann, D.J. Hicklin, D.L. Ludwig, J. Baselga, N. Rosen, mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt, Cancer Res. 66 (2006) 1500–1508.
[10] S.V. Bhagwat, P.C. Gokhale, A.P. Crew, A. Cooke, Y. Yao, C. Mantis, J. Kahler, J. Workman, M. Bittner, L. Dudkin, D.M. Epstein, N.W. Gibson, R. Wild, L.D. Arnold, P.J. Houghton, J.A. Pachter, Preclinical characterization of OSI-027, a potent and selective inhibitor of mTORC1 and mTORC2: distinct from rapamycin, Mol. Cancer Ther. 10 (2011) 1394–1406.
[11] I. Kim, K. Kim, J. Choi, A direct approach to 5-hydroxybenzofurans via a platinum-catalyzed domino rearrangement/5-endo-dig cyclization reaction of quinols, J. Org. Chem. 74 (2009) 8492–8495.
[12] J.F. Cheng, B.N. Nguyeng, X. Liu, G.D. Lopaschuk, J. Dyck, Heterocyclic Compounds Useful as Malonyl-CoA Decarboxylase Inhibitors, US 20050026969, 2005.
[13] K. Yoshihara, D. Suzuki, Y. Susumu, Y. Hiroyoshi. M. Hisashi, S. Norio, Preparation of Guanidine Compounds as VAP-1 Inhibitors, PCT Int. Appl. WO 2012124696, 2012.