Synthesis and Biological Evaluation of 2-aroyl-4-phenyl-5-hydroxybenzofurans as a New Class of Antitubulin Agents

Romeo Romagnoli[1,*], Pier Giovanni Baraldi[1], Taradas Sarkar[2], Carlota Lopez Cara[1], Olga Cruz Lopez[1], Maria Dora Carrion[1], Delia Preti[1], Manlio Tolomeo[3], Jan Balzarini[4] and Ernest Hamel[2]

[1]Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy; [2]Toxicology and Pharmacology Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, USA; [3]Divisione di Ematologia e Servizio AIDS, Dipartimento di Oncologia, Policlinico "P. Giaccone", Università di Palermo, Palermo, Italy; [4]Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Abstract. Microtubules are among the most successful targets for development of compounds useful for anticancer therapy. Continuing our project to develop new small molecule antitumor agents, two new series of derivatives based on the 2-aroyl-4-phenylbenzofuran molecular skeleton were synthesized and evaluated for antiproliferative activity, inhibition of tubulin polymerization and cell cycle effects. SAR were elucidated with various substitutions on the benzoyl moiety at the 2-position of the benzofuran ring. The most promising compound in this series, the (5-hydroxy-4-phenylbenzofuran-2-yl)(4-methoxyphenyl)methanone derivative (**3d**), has significant growth inhibitory activity in the submicromolar range against the Molt4, CEM and HeLa cancer cell lines and interacts with tubulin by binding to the colchicine site. Exposure to **3d** led to the arrest of K562 cells in the G2-M phase of the cell cycle and to the induction of apoptosis.

Key Words: Microtubule, benzofuran, antiproliferative activity.

1. INTRODUCTION

There is considerable interest in the discovery and development of novel small molecules able to affect tubulin polymerization. Such compounds impair dynamic microtubule elements of the cell cytoskeleton that are responsible for the formation of the mitotic spindle and required for proper chromosomal separation during cell division [1-4].

It is widely known that numerous compounds containing the benzo[b]furan system, isolated from natural sources as well as synthetic agents, show cytostatic and/or cytotoxic activity [5-7]. The antitumor activity of benzofuran derivatives appears to be dependent on substitution at the heterocyclic furan ring rather than at the benzene moiety.

Hayakawa *et al*. demonstrated that several synthesized 6-phenyl-2-aroylbenzofuran derivatives with general structure **1** showed selective cytotoxicity against human tumorigenic cell line VA13 [8-10]. Xian *et al*. recently reported screening of a series of compounds with the 2-aryl benzo[b]furan skeleton [11]. Among them, the 2-(3-formyl-2-(4-hydroxy-3-methoxyphenyl)benzofuran-5-yl)ethyl acetate derivative **2** exhibited prominent cytoxic effects on a variety of tumor cell lines, with IC_{50} values of 5-18 μM.. The cytotoxic mechanism was related to inhibition of tubulin polymerization.

Inspired by these publications, in a continuation of our research on finding new antiproliferative compounds and exploring their mechanism of action, we designed and prepared two new series of benzofuran derivatives. These compounds have structures **3a-f** and **4a-f**, which are characterized by the presence of a benzene and a hydroxy or methoxy substituent at the 4- and 5-positions of the benzofuran moiety, respectively. We also investigated the biological activity of a series of molecules bearing one, two or three electron-donating methoxy groups on the benzoyl moiety at the 2-position of benzofuran system.

2. CHEMISTRY

Benzofuran derivatives **3a-f** and **4a-f** were synthesized as shown in Scheme **1**. The 2-aroyl-4-bromo-5-hydroxybenzo [b]furans **7a-f** were prepared in good yields, by condensation of 2-bromo-3-*t*-butyldimethylsilyloxy-6-hydroxybenzaldehyde **5** [12] with the respective α-bromo substituted acetophenones **6a-f** [13], followed by intramolecular cyclization, using potassium carbonate in refluxing acetone. The phenolic hydroxy group of **7a-f** was protected as *tert*-butyldimethylsilyl (TBDMS) ether by treatment with TBDMSCl and imidazole, to furnish derivatives **8a-f**. 4-Phenylbenzofurans **9a-f** were synthesized from TBDMS-protected phenols **7a-f** by a standard Suzuki cross-coupling reaction [14] with phenylboronic acid under heterogeneous conditions [$Pd(PPh_3)_4$, K_2CO_3] in refluxing toluene. After removal of the TBDMS protective group using tetra-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran (THF), the 4-phenyl-5-hydroxybenzofurans **3a-f** were obtained and converted into the corresponding 5-methoxy derivatives **4a-f** by treatment with methyl sulfate (Me_2SO_4) in refluxing THF.

3. RESULTS AND DISCUSSION.

Table **1** summarizes the antiproliferative effects of benzo[b]furan derivatives **3a-f** and **4a-f** against murine leu-

*Address correspondence to this author at the Dipartimento di Scienze Farmaceutiche, Università di Ferrara, *Via* Fossato di Mortara 17-19, I-44100 Ferrara, Italy; Tel: +39-(0)532-455303; Fax: +39-(0)532-455953;
E-mail: rmr@unife.it

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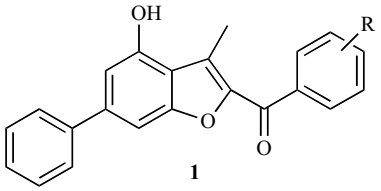
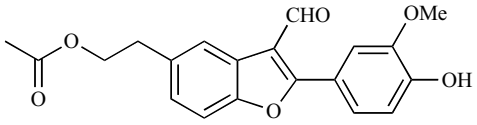


1

R=H, OMe, CF_3, F



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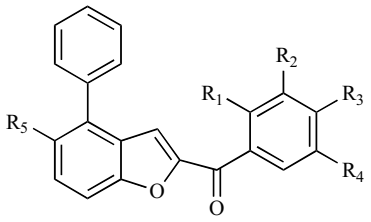


3a, $R_{1\text{-}4}$=H, R_5=OH
3b, R_1=OMe, $R_{2\text{-}4}$=H, R_5=OH
3c, R_2=OMe, $R_{1,\,3\text{-}4}$=H, R_5=OH
3d, R_3=OMe, $R_{1\text{-}2,\,4}$=H, R_5=OH
3e, $R_{2,4}$=OMe, $R_{1,3}$=H, R_5=OH
3f, R_1=H, $R_{2\text{-}4}$=OMe, R_5=OH
4a, $R_{1\text{-}4}$=H, R_5=OMe
4b, $R_{1,\,5}$=OMe, $R_{2\text{-}4}$=H
4c, $R_{2,\,5}$=OMe, $R_{1,\,3\text{-}4}$=H
4d, $R_{3,\,5}$=OMe, $R_{1\text{-}2,\,4}$=H
4e, $R_{2,\,4\text{-}5}$=OMe, $R_{1,3}$=H
4f, R_1=H, $R_{2\text{-}5}$=OMe

Chart (1). Chemical structure of benzofurans with general structure **1-4**.

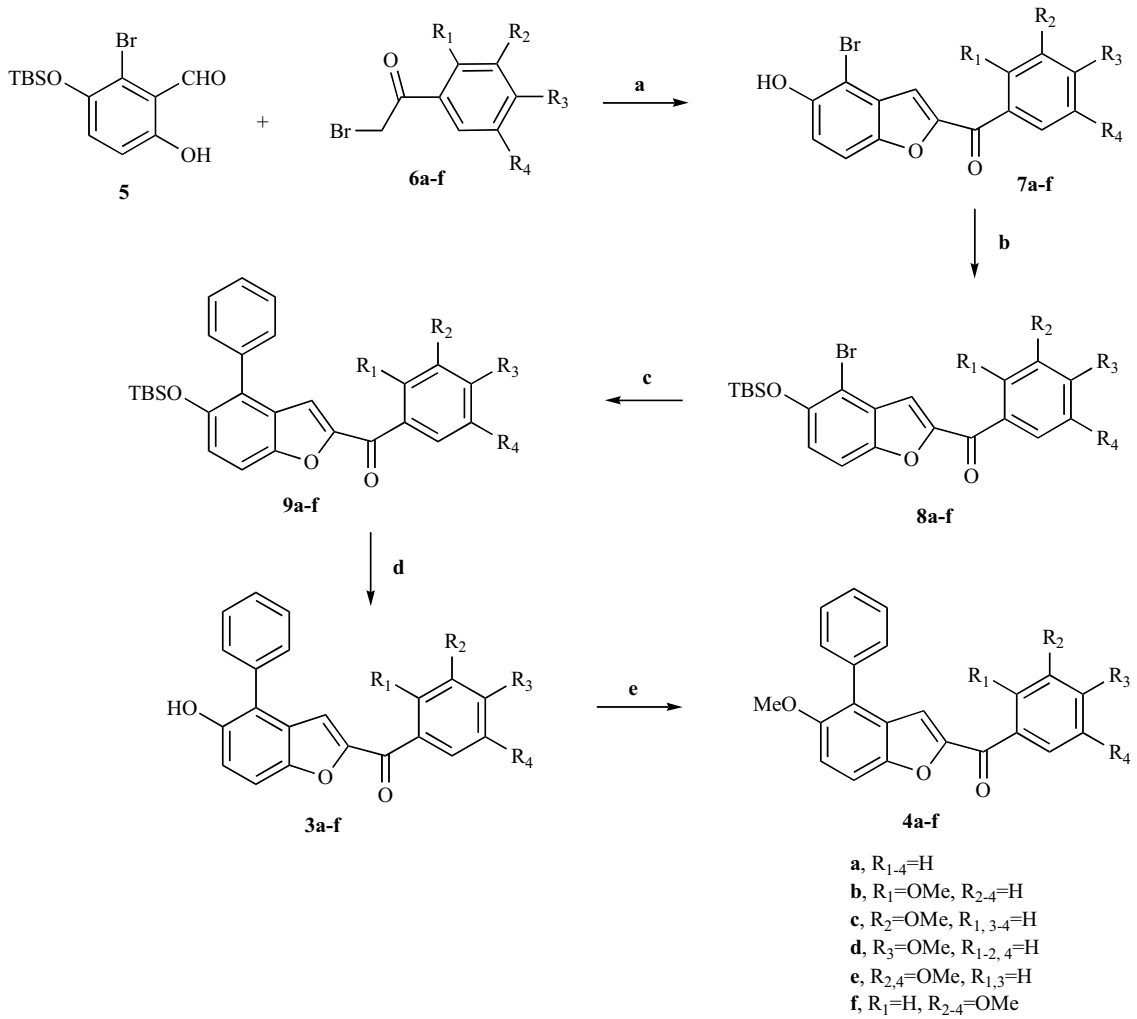
kemia (L1210), murine mammary carcinoma (FM3A), human T-lymphoblastoid (Molt/4 and CEM) and human cervical carcinoma (HeLa) cells, using Combretastatin A-4 (CA-4) as reference compound [15]. All new synthesized molecules displayed inhibitory activities lower to that of CA-4 against four cancer cell lines.

In general, the antiproliferative activities of the tested compounds were more pronounced against Molt/4, CEM and HeLa cells as compared with the two murine cell lines (Table **1**). Derivative **3d**, which had the greatest antiproliferative activity in this series, inhibited the growth of L1210, FM3A, Molt/4 CEM and HeLa cancer cell lines with an IC_{50} of 1.3, 1.9, 0.69, 0.88 and 0.51 μM, respectively. The cognate compound, **4d**, with a methoxy group instead of a hydroxy group at the C-5 position of the benzofuran system, was nearly as active, with IC_{50}'s of 2.8, 8.4, 1.4, 1.5 and 1.3 μM, respectively in the five cell lines.In general, except for compounds **3c** and **3d**, replacing the phenolic hydroxy group at the C-5 position of benzofuran system (compounds **3a-f**) with a methoxy moiety (derivatives **4a-f**) led to a reduction in antiproliferative activity.

We also evaluated the biological effects caused by electron-releasing methoxy groups on the benzoyl moiety at the 2-position of the benzofuran ring. While the 2-benzoyl benzofuran derivative **3a** showed weak activity (IC_{50} > 30 μM), the antiproliferative effects were markedly enhanced by a single methoxy group *para* to the carbonyl moiety (**3d** and **4d**). A lesser effect, especially in the 5-methoxy series, also occurred with a single methoxy group in the *meta* position (**4d**). A single methoxy group *ortho* to the carbonyl moiety also appeared to enhance activity in some lines in the 5-hydroxy series (cf. **3b** with **3a**).With two *meta* methoxy groups (**3e** and **4e**) relative activity was dependent on the substituent at C-5 in the benzofuran system:with the C-5 hyroxyl group, the dimethoxy compound was more active than the monomethoxy compound (cf. the more active **3e** with **3c**), while, with the C-5 methoxyl group, **4c**, with a single *para* methoxy group was much more active than **4e** with two methoxy groups. The two trimethoxy derivative, **3f** and **4f**, were inactive, except with HeLa cells.

To investigate whether the antiproliferative activities of these compounds were related to interaction with the microtubule system, compound **3d** was evaluated for *in vitro* inhibition of tubulin polymerization and for inhibitory effects on the binding of [^{3}H]colchicine to tubulin (in the latter assay, the compound and tubulin were examined at a concentration of 1 μM with the colchicine at 5 μM) [16, 17] For comparison, CA-4 was examined in contemporaneous experiments. In the tubulin polymerization assay, compound **3d** was 2-fold less active than CA-4, with IC_{50} values of 2.8 and 1.4 μM, respectively.

In the colchicine binding studies, 39% inhibition occurred with **3d** at 1 μM and the colchicine at 5 μM. Again, **3d** was less potent than CA-4, which in these experiments inhibited colchicine binding by 87%.



Reagents: **a**: K_2CO_3, acetone, reflux, 18h; **b**: TBDMSCl, imidazole,CH_2Cl_2, rt; **c**: $C_6H_5B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, 18 h; **d**: TBAF, THF, rt, 1h; **e**: Me_2SO_4. THF, reflux.

Scheme 1.

Because molecules exhibiting activity on tubulin binding should cause alteration of cell cycle parameters, with preferential G2-M blockade, the effects on the cell cycle of **3d** was examined by flow cytometry after staining cells with propidium iodide. K562 cells (acute myeloblastic leukemia) were exposed for 24 h to a 2 μM concentration of **3d**. As shown in Fig. **1B**, compound **3d** treatment induced an accumulation of K562 cells in G2-M, with a simultaneous decrease of cells in S and G0-G. In addition, **3d** induced apoptosis as shown by the appearance of a sub-G0-G1 peak.

This data confirms that **3d** acts selectively on the G2-M phase of the cell cycle, as expected for an inhibitor of tubulin assembly.

In conclusion, we have discovered a new class of simple synthetic inhibitors of tubulin polymerization, based on a 2-benzoyl-4-phenyl-5-hydroxybenzofuran molecular skeleton. The greatest activity occurred when the methoxy group was located at the *para* position of the 2-benzoyl moiety, the least when located at the *meta* position. Among the tested compounds, the best results for antiproliferative activity were obtained with the (5-hydroxy-4-phenylbenzofuran-2-yl) (4-methoxyphenyl)methanone derivative (**3d**), which was active at sub-micromolar concentrations against Molt/4, CEM and HeLa cancer cell lines. We identified tubulin as the molecular target of this compound, since it inhibited tubulin assembly and the binding of colchicine to tubulin. We also showed by flow cytometry that **3d** had cellular effects typical of agents that bind to tubulin, causing accumulation of K562 cells in the G2-M phase of the cell cycle and a substantial increase in the number of apoptotic cells. These results suggest that compound **3d** is worthy of further investigation and offer new possibilities for further explorations to improve potency.

4. EXPERIMENTAL PROTOCOLS

4.1. Chemistry

^{1}H-NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane as internal standard, and the spectra were recorded in appropriate deuterated solvents, as indicated. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned

Table 1. In Vitro Inhibitory Effects of CA-4, Compounds 3a-f and 4a-f Against the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), Human T-Lymphocyte (Molt/4 and CEM) and Human Cervical Carcinoma (HeLa) cells

Compound	IC_{50} (μM)				
	L1210	FM3A/0	Molt4/C8	CEM/0	Hela
3a	37±5	37±18	38±4	34±7	35±2
3b	>50	>50	13±1	19±14	24±7
3c	38±3	38±2	47±14	35±10	27±32
3d	1.3±1.0	1.9±0.0	0.69±0.61	0.88±0.63	0.51±0.32
3e	19±4	30±2	12±1	9.6±1.7	15±8
3f	>50	>50	>50	>50	31±18
4a	>50	>50	>50	>50	>50
4b	>50	>50	>50	>50	>50
4c	10±4	13±0	4.8±1.1	5.4±0.1	5.4±1.7
4d	2.8±0.42	8.4±1.0	1.4±0.31	1.5±0.11	1.3±0.40
4e	>50	>50	>50	>50	>50
4f	>50	>50	>50	>50	25±16
CA-4	0.0032±0.0007	0.042±0.003	0.021±0.0008	0.0018±0.0004	n.d.

[a]IC_{50}= compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose-response curves of at least three independent experiments.

n.d.= not determined.

structures. Elemental analyses were conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara. Analyses indicated by the symbols of the elements or functions were within ± 0.4% of the theoretical values. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Merck plates) and visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230-400 mesh silica gel and the indicated solvent system. Organic solutions were dried over anhydrous Na_2SO_4.

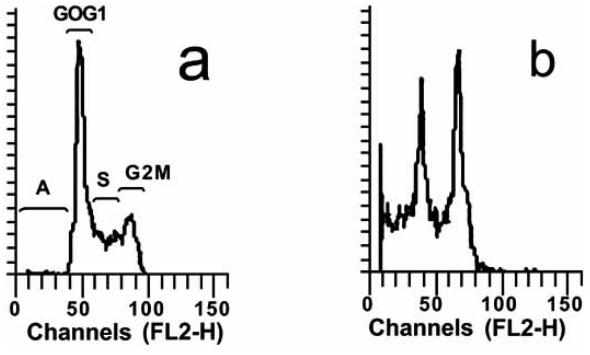


Fig. (1). Effect of compound **3d** (**b**) on DNA content/cell following treatment of K562 cells for 24 h. The cells were cultured without compound (control, panel **a**) or with compound **3d** used at a cytostatic concentration. Cell cycle distribution was analysed by the standard propidium iodide procedure. Control cells at the sub-G0-G1 (labeled A), G0-G1, S, and G2-M phases of the cell cycle are indicated in panel **a**.

4.2. General Procedure A for the Synthesis of 7a-f

To a stirred solution of **5** (1 mmol) in dry acetone (15 mL) was added the α-bromoacetophenone **6a-f** (1 mmol) and anhydrous potassium carbonate (276 mg, 2 mmol), and the reaction mixture was refluxed for 18 h. After cooling, the solvent was evaporated, and the residue dissolved in a mixture of dichloromethane (15 mL) and water (5 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to obtain a residue purified by flash column chromatography.

4.2.1. (4-Bromo-5-hydroxybenzofuran-2-yl)(phenyl)methanone (7a)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **7a** as a yellow solid (52% yield); mp 128-130°C. ^{1}H-NMR ($CDCl_3$) δ: 5.47 (s, 1H), 7.21 (d, J=8.6 Hz, 1H), 7.48 (m, 6H), 8.13 (d, J=8.6 Hz, 1H).

4.2.2. (4-Bromo-5-hydroxybenzofuran-2-yl)(2-methoxyphenyl)methanone (7b)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **7b** as a yellow solid (67% yield); mp 129-131°C. ^{1}H-NMR ($CDCl_3$) δ: 3.81 (s, 3H), 5.32 (s, 1H), 7.04 (m, 2H), 7.26 (m, 1H), 7.49 (m, 4H).

4.2.3. (4-Bromo-5-hydroxybenzofuran-2-yl)(3-methoxyphenyl)methanone (7c)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **7c** as a yellow solid (63% yield); mp 120-122°C. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (s, 3H), 5.53 (s, 1H), 7.22 (m, 2H), 7.49 (m, 4H), 7.66 (d, J=7.6 Hz, 1H).

4.2.4. (4-Bromo-5-hydroxybenzofuran-2-yl)(4-methoxyphenyl)methanone (7d)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **3d** as a white solid (62% yield); mp 164-165°C. ^{1}H-NMR ($CDCl_3$) δ: 3.92 (s, 3H), 5.54 (s, 1H), 7.01 (d, J=6.8 Hz, 2H), 7.22 (d, J=8.8 Hz, 1H), 7.44 (d, J=8.8 Hz, 1H), 7.52 (s, 1H), 8.13 (d, J=6.8 Hz, 2H).

4.2.5. (4-Bromo-5-hydroxybenzofuran-2-yl)(3,5-dimethoxyphenyl)methanone (7e)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **7e** as a yellow solid (48% yield); mp 185-187 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 6H), 5.52 (s, 1H), 6.74 (s, 1H), 7.16 (s, 2H), 7.19 (d, J=8.8 Hz, 1H), 7.46 (d, J=8.8 Hz, 1H), 7.48 (s, 1H).

4.2.6. (4-Bromo-5-hydroxybenzofuran-2-yl)(3,4,5-trimethoxyphenyl)methanone (7f)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent, furnished **7f** as a white solid (58% yield); mp 196-198 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.96 (s, 3H), 3.97 (s, 6H), 5.52 (s, 1H), 7.24 (d, J=9.2 Hz, 1H), 7.34 (s, 2H). 7.47 (s, 1H), 7.48 (d, J=9.2 Hz, 1H).

4.3. General procedure B for the synthesis of *8a-f*

To a solution of phenol **7a-f** (1 mmol) in CH_2Cl_2 (10 mL) were added TBDMSCl (1.2 mmol, 1.2 equiv., 185 mg) and imidazole (2.5 mmol, 2.5 equiv., 170 mg). The mixture was stirred at rt for 18 h, after which it was diluted with dichloromethane (10 mL), washed with water (5 mL) and brine (5 mL) and the organic phase was dried over Na_2SO_4. After concentration *in vacuo*, the residue was purified by flash chromatography on silica gel.

4.3.1. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(phenyl)methanone (8a)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **8a** as a colorless oil (68% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.26 (s, 6H), 1.07 (s, 9H), 7.04 (d, J=8.8 Hz, 1H), 7.44 (m, 6H), 8.05 (d, J=8.8 Hz, 1H).

4.3.2. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(2-methoxyphenyl)methanone (8b)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **8b** as a yellow oil (72% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.25 (s, 6H), 1.06 (s, 9H), 3.83 (s, 3H), 7.06 (m, 4H), 7.28 (s, 1H), 7.44 (d, J=7.8 Hz, 1H), 7.60 (d, J=7.8 Hz, 1H).

4.3.3. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(3-methoxyphenyl)methanone (8c)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **8c** as a yellow oil (76% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.26 (s, 6H), 1.07 (s, 9H), 3.90 (s, 3H), 7.03 (d, J=8.4 Hz, 1H), 7.17 (dd, J=8.4 and 2.8 Hz, 1H), 7.42 (d, J=7.8 Hz, 1H), 7.52 (d, J=7.8 Hz, 1H), 7.52 (m, 2H), 7.66 (d, J=7.8 Hz, 1H).

4.3.4. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(4-methoxyphenyl)methanone (8d)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **8d** as a yellow oil (81% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.27 (s, 6H), 1.07 (s, 9H), 3.92 (s, 3H), 7.01 (m, 3H), 7.47 (m, 2H), 8.08 (d, J=9.0 Hz, 2H).

4.3.5. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(3,5-dimethoxyphenyl)methanone (8e)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **4e** as a white solid (66% yield), mp 119-120 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.26 (s, 6H), 1.07 (s, 9H), 3.87 (s, 6H), 6.72 (s, 1H), 7.04 (d, J=9.0 Hz, 1H), 7.14 (s, 2H), 7.46 (d, J=9.0 Hz, 1H), 7.48 (s, 1H).

4.3.6. (4-Bromo-5-t-butyldimethylsilyloxybenzofuran-2-yl)(3,4,5-trimethoxyphenyl)methanone (8f)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **8f** as a yellow oil (86% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.27 (s, 6H), 1.07 (s, 9H), 3.96 (s, 3H), 3.97 (s, 6H), 7.06 (d, J=9.0 Hz, 1H), 7.32 (s, 2H), 7.43 (d, J=9.0 Hz, 1H), 7.50 (s, 1H).

4.4. General procedure C (Suzuki coupling) for the synthesis of compounds 9a-f

A mixture of derivatives **4a-f** (0.5 mmol), potassium carbonate (104 mg, 0.75 mmol, 1.5 equiv.), the appropriate aryl boronic acid (1 mmol, 2 equiv.) and tetrakis (triphenylphosphine)palladium (13.5 mg, 0.012 mmol) in dry toluene (10 mL) was stirred at 100 °C under nitrogen for 18 h, cooled to ambient temperature and evaporated in vacuo. The residue was dissolved with EtOAc (30 mL), and the resultant solution was washed sequentially with 5% $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried, filtered and evaporated, and the residue was purified by flash chromatography on silica gel.

4.4.1. (4-Phenyl-5-t-butyldimethylsilyloxybenzofuran-2-yl)(phenyl)methanone (9a)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **9a** as a colorless oil (63% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.28 (s, 6H), 1.09 (s, 9H), 7.02 (d, J=8.8 Hz, 1H), 7.38 (m, 11H), 8.07 (d, J=8.8 Hz, 1H).

4.4.2. (4-Phenyl-5-t-butyldimethylsilyloxybenzofuran-2-yl)(2-methoxyphenyl)methanone (9b)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, fur-

nished **5b** as a yellow oil (72% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.10 (s, 6H), 0.81 (s, 9H), 3.81 (s, 3H), 7.02 (d, J=8.4 Hz, 1H), 7.11 (d, J=8.4 Hz, 1H), 7.18 (s, 1H), 7.47 (m, 9H).

4.4.3. (4-Phenyl-5-t-butyldimethylsilyloxybenzofuran-2-yl) (3-methoxyphenyl)methanone (9c)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **9c** as a yellow oil (77% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.10 (s, 6H), 0.81 (s, 9H), 3.87 (s, 3H), 7.07 (d, J=8.4 Hz, 1H), 7.49 (m, 11H).

4.4.4. (4-Phenyl-5-t-butyldimethylsilyloxybenzofuran-2-yl) (4-methoxyphenyl)methanone (9d)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **9d** as a yellow oil (62% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.11 (s, 6H), 0.82 (s, 9H), 3.89 (s, 3H), 6.96 (d, J=9.0 Hz, 2H), 7.09 (d, J=8.6, 1H), 7.44 (m, 7H), 8.06(d, J=9.0 Hz, 2H).

4.4.5. (4-Phenyl-5-t-butyldimethylsilyloxybenzofuran-2-yl) (3,5-dimethoxyphenyl)methanone (9e)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **9e** as a yellow oil (91% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.10 (s, 6H), 0.81 (s, 9H), 3.84 (s, 6H), 6.68 (s, 1H), 7.11 (d, J=9.0 Hz, 1H), 7.12 (s, 2H), 7.44 (m, 7H).

4.4.6. (4-Phenyl -5-t-butyldimethylsilyloxybenzofuran-2-yl) (3,4,5-trimethoxyphenyl)methanone (9f)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **9f** as a yellow oil (85% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.10 (s, 6H), 0.83 (s, 9H), 3.92 (s, 3H), 3.94 (s, 6H), 7.09 (d, J=8.8 Hz, 1H), 7.26 (s, 2H), 7.49 (m, 7H).

4.5. General procedure D for the synthesis of 3a-f

To a solution of **9a-f** (2 mmol) dissolved in THF (10 mL), TBAF (6 mmol, 3 equiv.) was added, and the mixture was stirred at rt for 1 h. The solution was concentrated under vacuo, diluted with DCM (15 mL), washed with water (3x5 mL) and brine (5 mL) and dried over Na_2SO_4. After concentration, the residue was purified by flash chromatography on silica gel.

4.5.1. (5-Hydroxy-4-phenylbenzofuran-2-yl)(phenyl)methanone (3a)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:9 (v:v) as eluent, furnished **3a** as a white solid (73% yield), mp 112-114 °C. ^{1}H-NMR ($CDCl_3$) δ: 5.22 (bs, 1H), 7.19 (d, J=8.4 Hz, 1H), 7.38 (m, 11H), 7.98 (d, J=8.8 Hz, 1H). Anal. $C_{21}H_{14}O_3$ (C, H).

4.5.2. (5-Hydroxy-4-phenylbenzofuran-2-yl)(2-methoxyphenyl)methanone (3b)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **3b** as a yellow solid (96% yield), mp 140-142 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.80 (s, 3H), 5.23 (s, 1H), 6.98 (d, J=8.4 Hz, 1H), 7.11(d, J=7.6, 1H), 7.20 (s, 1H), 7.50 (d, 9H). Anal. $C_{21}H_{14}O_3$ Anal. $C_{22}H_{16}O_4$ (C, H).

4.5.3. (5-Hydroxy-4-phenylbenzofuran-2-yl)(3-methoxyphenyl)methanone (3c)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **3c** as a yellow solid (86% yield), mp 148-150 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 3H), 5.22 (s, 1H), 7.14 (m, 1H), 7.18 (d, J=9.0, 1H), 7.26 (d, J=9.0 Hz, 1H), 7.41 (t, J=7.8 Hz, 1H), 7.54 (d, 8H). Anal. $C_{22}H_{16}O_4$ (C, H).

4.5.4. (5-Hydroxy-4-phenylbenzofuran-2-yl)(4-methoxyphenyl)methanone (3d)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **3d** as a yellow solid (83% yield), mp 153-155 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.89 (s, 3H), 5.25 (s, 1H), 6.98 (d, J=8.6 Hz, 2H), 7.17(d, J=9.2 Hz, 1H), 7.33 (s, 1H), 7.54 (m, 6H), 8.06 (d, J=8.6 Hz, 2H). Anal. $C_{22}H_{16}O_4$ (C, H).

4.5.5. (5-Hydroxy-4-phenylbenzofuran-2-yl)(3,5-dimethoxyphenyl)methanone (3e)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent, furnished **3e** as a yellow solid (66% yield), mp 197-199 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.64 (s, 6H), 5.24 (bs, 1H), 6.68 (s, 1H), 7.12 (d, J=9.0 Hz, 1H), 7.23 (s, 2H), 7.52 (m, 7H). Anal. $C_{23}H_{18}O_5$ (C, H).

4.5.6. (5-Hydroxy-4-phenylbenzofuran-2-yl)(3,4,5-trimethoxyphenyl)methanone (3f)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **3f** as a white solid (91% yield), mp 224-226 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.91 (s, 3H), 3.94 (s, 6H), 5.32 (bs, 1H), 7.02 (s, 1H), 7.19 (d, J=8.8 Hz, 1H), 7.33 (s, 2H), 7.50 (m, 6H). Anal. $C_{24}H_{20}O_6$ (C, H).

4.6. General procedure E for the synthesis of 4a-f

To a suspension of compound **3a-f** (1 mmol) and anhydrous K_2CO_3 (1 mmol, 138 mg, 1 equiv.) in THF (5 mL) was added Me_2SO_4 (1 mmol, 126 mg, 90 μL), and the resulting solution was refluxed for 4 h. After this period, the solution was filtered, the filtrate evaporated to dryness and the residue extracted with a mixture of EtOAc (20 mL) and water (5 mL). The organic phase was washed with brine (5 mL), dried and concentrated in vacuo, and the residue was purified by flash chromatography on silica gel.

4.6.1. (5-Methoxy-4-phenylbenzofuran-2-yl)(phenyl)methanone (4a)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **4a** as a white solid (81% yield), mp 124-126 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (s, 3H), 7.10 (d, J=8.6 Hz, 1H), 7.42 (m, 11H), 8.028 (d, J=8.6 Hz, 1H). Anal. $C_{22}H_{16}O_3$ (C, H).

4.6.2. (5-Methoxy-4-phenylbenzofuran-2-yl)(2-methoxyphenyl)methanone (4b)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **4b** as a white solid (68% yield), mp 156-158 °C. ^{1}H-

NMR ($CDCl_3$) δ: 3.78 (s, 3H), 3.81 (s, 3H), 7.02 (d, J=8.4 Hz, 1H), 7.12 (d, J=7.6 Hz, 1H), 7.20 (s, 1H), 7.50 (d, 9H). Anal. $C_{23}H_{18}O_4$ (C, H).

4.6.3. (5-Methoxy-4-phenylbenzofuran-2-yl)(3-methoxyphenyl)methanone (4c)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent, furnished **4c** as a white solid (64% yield), mp 158-160 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 3H), 3.90 (s, 3H), 7.08 (m, 1H), 7.20 (d, J=9.0 Hz, 1H), 7.28 (d, J=9.0, 1H), 7.44 (t, J=7.8 Hz, 1H), 7.59 (d, 8H). Anal. $C_{23}H_{18}O_4$ (C, H).

4.6.4. (5-Methoxy-4-phenylbenzofuran-2-yl)(4-methoxyphenyl)methanone (4d)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **4d** as a yellow solid (83% yield), mp 117-119 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.82 (s, 3H), 3.89 (s, 3H), 6.98 (d, J=9.0 Hz, 2H), 7.22(d, J=9.2 Hz, 1H), 7.38 (s, 1H), 7.54 (m, 6H), 8.05 (d, J=9.0 Hz, 2H). Anal. $C_{23}H_{18}O_4$ (C, H).

4.6.5. (5-Methoxy-4-phenylbenzofuran-2-yl)(3,5-dimethoxyphenyl)methanone (4e)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **4e** as a yellow oil (54% yield). ^{1}H-NMR ($CDCl_3$) δ: 3.82 (s, 3H), 3.84 (s, 6H), 6.69 (s, 1H), 7.12 (s, 2H), 7.24 (d, J=9.2 Hz, 1H), 7.52 (m, 7H). Anal. $C_{24}H_{20}O_5$ (C, H).

4.6.6. (5-Methoxy-4-phenylbenzofuran-2-yl)(3,4,5-trimethoxyphenyl)methanone (4f)

The crude residue, purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent, furnished **4f** as a white solid (87% yield), mp 150-152 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.83 (s, 3H), 3.92 (s, 3H), 3.95 (s, 6H), 7.13 (s, 1H), 7.23 (d, J=9.0 Hz, 1H), 7.42 (s, 2H), 7.53 (m, 6H). Anal. $C_{25}H_{22}O_6$ (C, H).

5. BIOLOGICAL EVALUATION

5.1. Inhibition of Tumor Cell Growth

Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte Molt 4 and CEM and human cervical carcinoma (HeLa) cells were suspended at 300,000-500,000 cells/mL of culture medium, and 100 µL of a cell suspension was added to 100 µL of an appropriate dilution of the test compounds in wells of microtiter plates. After incubation at 37 °C for two (L1210 and FM3A) or three (Molt 4 and CEM) days, cell number was determined using a Coulter counter. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%.

5.2. Effects on Tubulin Polymerization

To evaluate the effect of the compounds on tubulin assembly *in vitro* [16], varying concentrations were preincubated with 10 µM tubulin in glutamate buffer at 30 ˚C and then cooled to 0 ˚C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed to 30 °C, and the assembly of tubulin was observed turbidimetrically. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min at 30 °C. Inhibition of the binding of [^{3}H]colchicine to tubulin was determined as described previously [17].

5.3. Flow Cytometric Analysis of Cell Cycle Distribution

The effects of the most active compounds of the series on cell cycle distribution were studied with K562 cells (myeloblastic leukemia) by flow cytometric analysis after staining with propidium iodide. Cells were exposed 24 h to each compound used at a concentration corresponding to the IC_{50} determined after a 24 h incubation. After treatment, the cells were washed once in ice-cold PBS and resuspended at 1×10^6 per mL in a hypotonic fluorochrome solution containing propidium iodide (Sigma) at 50 µg/mL in 0.1% sodium citrate plus 0.03% (v/v) nonidet P-40 (Sigma). After a 30 min incubation, the fluorescence of each sample was analyzed as single-parameter frequency histograms by using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). The distribution of cells in the cell cycle was analyzed with the ModFit LT3 program (Verity Software House, Inc.).

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REFERENCES

[1] Li, Q.; Sham, H.L. *Expert Opin. Ther. Pat.,* **2002**, *12*, 1663.
[2] Honore, S.; Pasquier, E.; Braguer, D. U. *Cell. Mol. Life. Sci.*, **2005**, *62*, 3039.
[3] Pellegrini, F.; Budman, D.R. *Cancer Invest.,* **2005**, *23*, 264.
[4] Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. *Curr. Opin. Invest. Drugs,* **2006**, *7*, 522.
[5] Donnelly, D.M.X.; Meegan, M.J. In *Comprehensive Heterocyclic Chemistry,* Katritky, A.R.; Rees, C.W., Eds., Pergamon: Oxford, **1984**, Vol. *4*, pp 657-712.
[6] Cagniant, P.; Cagniant, D. In *Advancesin Heterocyclic Chemistry*, Katritky, A.R., Boulton, A.J., Eds., Academic: New York, **1975**: Vol. *18*, pp 343-473.
[7] Kossakowski, J.; Ostrowska, K.; Hejchman, E.; Wolska, I. *Il Farmaco,* **2005**, *60*, 519.
[8] Hayakawa, I.; Shiota, R.; Agatsuma, T.; Furukawa, H.; Naruto, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 455.
[9] Hayakawa, I.; Shiota, R.; Agatsuma, T.; Furukawa, H.; Naruto, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 4383.
[10] Hayakawa, I.; Shiota, R.; Agatsuma, T.; Furukawa, S.; Sugano, Y. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 3411.
[11] Yang, H.; Pang, J.-Y.; Cai, Y.-C.; Xu, Z.-L.; Xian, L.-J. *J. Pharm. Pharmacol.,* **2006**, *58*, 1281.
[12] Hu, Y.; Li, C.; Kulkarni, B. A.; Strobel, G.; Lobkovsky, E.; Torczynski, R. M.; Porco, J. A. *Org. Lett.,* **2001**, *3*, 1649.
[13] **6a-d** are commercially available, **6e** and **6f** were synthesized following the procedure reported in the article: Dominianni, S.J.; Yen, T.T. *J. Med. Chem.,* **1989**, *32*, 2301.
[14] Shieh, W. C.; Carlson, J. A. *J. Org. Chem*., **1992**, *57*, 379.
[15] Pettit, G.R.; Singh, S.B.; Hamel, E.; Lin, C.M.; Alberts, D.S.; Garcia-Kendall, D. *Experentia*, **1989**, *45*, 209.
[16] Hamel, E. *Cell Biochem. Biophys.,* **2003**, *38*, 1.
[17] Verdier-Pinard, P.; Lai J.-Y.; Yoo, H.-D.; Yu, J.; Marquez, B.; Nagle D.G.; Nambu, M.; White, J.D.; Falck, J.R.; Gerwick, W.H.; Day, B.W.; Hamel, E.. *Mol. Pharmacol.,* **1998**, *53*, 62.