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Synthesis and Evaluation of Haloacetyl, α -Bromoacryloyl and Nitrooxyacetyl Benzo[*b*]furan and Benzo[*b*]thiophene Derivatives as Potent Antiproliferative Agents Against Leukemia L1210 and K562 Cells

Romeo Romagnoli^{*,1}, Pier Giovanni Baraldi¹, Maria Dora Carrion¹, Carlota Lopez Cara¹, Alberto Casolari¹, Ernest Hamel², Enrica Fabbri³, and Roberto Gambari³

¹ Dipartimento di Scienze Farmaceutiche, Università di Ferrara, 44100 Ferrara, Italy

² Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, USA

³ BioPharmaNet, Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, 44100 Ferrara, Italy

Abstract

Identification of novel and selective anticancer agents remains an important and challenging goal in pharmacological research. In search of new compounds with strong antiproliferative activity and simple molecular structure, we have synthesized three different series of compounds in which different substituents were linked to the 3-amino position of the 2-(3', 4', 5'-trimethoxybenzoyl)-benzo[*b*]furan or benzo[*b*]thiophene ring system. These substituents, corresponding to acetyl/ haloacetyl, α -bromoacryloyl and nitrooxyacetyl moieties had different electrophilic properties. The benzoheterocycle parent structures were selected because of their reported bioactivities. Compounds bearing a methoxy group at the 6-position of the benzo[*b*]furan skeleton, were identified as potent antiproliferative agents against the human chronic myelogenous K562 and murine L1210 leukemia cell lines. Comparison of positional isomers indicated that moving the methoxy group from the 6- to the 5- or 7-position yielded inactive compounds. The effects of a selected series of compounds on cell cycle progression correlated well with their strong antiproliferative activity and inhibition of tubulin polymerization. The analysis of structure-activity relationships observed in the series of compounds described here may represent a platform for the design of more active molecules.

Keywords

Benzo[*b*]thiophene; Benzo[*b*]furan; Tumor cell growth; Antiproliferative agents

1. INTRODUCTION

Cancer is a major worldwide problem and the second leading cause of mortality in developed countries [1]. Since many of the current treatments have problems with toxicity and drug resistance, there is a strong demand for the discovery and development of effective

* 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; rmr@unife.it.

new cancer therapies [2]. In particular, great efforts have been made in past years in the search of new compounds for the treatment of leukemia [3]. Leukemia is one of the most common hematologic malignancies and an important cause of human death. In the treatment of leukemia patients, chemotherapy, radiotherapy and bone marrow transplantation are all used [4,5]. Leukemia therapy with anticancer compounds is based on cell growth inhibition, induction of cell death through apoptosis or induction of leukemic blast cell differentiation. Among the side effects of commonly used drugs are retinoic acid syndrome, pyomyositis, Sweet's syndrome, vasculitis, hypercalcemia, bone marrow necrosis and fibrosis, erythema nodosum, granulomatous proliferation and pulmonary complications [6,7]. Therefore, discovering novel, less toxic anti-leukemia agents is very important.

The microtubule system of eukaryotic cells is a critical element in a variety of fundamental cellular processes, such as cell division, formation and maintenance of cell shape, regulation of motility, cell signaling, secretion and intracellular transport [8]. Research oriented toward the discovery of new generation agents for cancer chemotherapy has identified microtubules as one of the most successful cellular targets [9].

Among these, 3-(haloacetamido) benzoylurea derivatives with general structure **1** were shown to be tubulin ligands that inhibit the polymerization of tubulin and cause apoptosis. In a preliminary structure-activity relationship (SAR) analysis, antiproliferative activity increased with increasing size of the halogen substituent, following the order I>Br>Cl. In addition, the presence of the haloacetamido chain at the *meta*-position of the benzene ring was essential for activity, as was the presence of the urea moiety [10,11]. The bioisosteric replacement of benzene with the bioisosteric thiophene ring furnished a series of compounds with IC₅₀ values ranging from 1.13 to 3.4 μ M against human leukemia CEM cells, while the introduction of a furan ring resulted in a loss of cytotoxicity, with IC₅₀ values between 4.05 and 16.7 μ M.

We have previously reported that the 2-(3',4',5'-trimethoxybenzoyl)-3-amino benzo[*b*]furan and benzo[*b*] thiophene molecular skeletons are the core structure of a series of molecules with general formula **2**. Such compounds inhibited tumor cell growth and tubulin polymerization by binding to the colchicine site of tubulin and caused G2-M phase arrest of the cell cycle [12,13]. In these compounds, the C-5, C-6 and C-7 substitution pattern with a methyl or a methoxy group plays an essential role in the biological potency.

Since the 2-(3',4',5'-trimethoxybenzoyl)-3-amino benzo[*b*]furan and benzo[*b*]thiophene structures were demonstrated to be essential for bioactivity, we retained these moieties throughout the present investigation, and we examined the anticancer effects of various acyl substituents, with different electrophilic nature, linked to the amino group at the 3-position of these benzoheterocycles. This furnished three different series of compounds.

In the first series, which is structurally related to derivatives with general formula **1**, we focused the study on the replacement of the benzoylurea urea moiety with the 2-(3',4',5'-trimethoxybenzoyl) benzo[*b*]furan or benzo[*b*] thiophene ring to furnish the 3-haloacetamido derivatives **3a-p** and **3q-ab**, respectively.

The second series was represented by the organic nitrates **4a-d**, characterized by the incorporation of the nitrooxyacetamido group as a nitric oxide (NO) releasing moiety. The rationale behind the preparation of this class of compounds is based on the property of NO as an antiproliferative agent [14]. NO exhibits a cytotoxic effect and enhances the cytotoxic effect of anticancer drugs due to its ability to influence various aspects of tumor biology, including modulation of cell growth, apoptosis, differentiation and tumor-induced immunosuppression [15,16].

The last third series of molecules **5a-g** was characterized by the presence of an α -bromoacryloyl alkylating moiety of low chemical reactivity, an unusual feature for cytotoxic compounds. In fact, α -bromoacrylic acid is not *per se* cytotoxic (IC₅₀ for L1210 cells being greater than 120 μ M) [17].

The reactivity of the α -bromoacryloyl moiety has been hypothesized to be based on a first-step Michael-type nucleophilic attack, followed by a further reaction of the former vinylic bromo substituent α to the carbonyl, leading successively either to a second nucleophilic substitution or to beta elimination [18].

We evaluated these three series of compounds for their antiproliferative activity against the murine L1210 and human chronic myelogenous K562 leukemia cell lines. Structure-activity relationships (SAR) were examined with electron-donating methyl and methoxy substitution at the 5-, 6- and 7-positions either of the benzo[*b*]furan and benzo[*b*]thiophene nucleus.

2. MATERIALS AND METHODS

2.1 Chemistry

2-Acetamido benzo[*b*]furan and benzo[*b*]thiophene derivatives **3a-ab**, **4a-d** and **5a-g** were synthesized following the strategy reported in Scheme 1. Acetylating or haloacetylating the 3-amino group of **2a-g** [12,13] with acetyl chloride, chloroacetyl chloride or bromoacetyl chloride in the presence of pyridine provided the (halo)acetamide derivatives **3aeimquy**, **3bfjnrvtz** and **3cgkoswaa**; respectively. The iodoacetyl derivatives **3dhptxab** were prepared from the bromoacetyl derivatives **3cgkoswaa** by an exchange reaction using sodium iodide in *N,N*-dimethylacetamide. The bromoacetamido derivatives **3c**, **3s**, **3w** and **3aa** were converted into the corresponding nitrooxy acetamido derivatives **4a-d** by treatment with silver nitrate (AgNO₃) in refluxing tetrahydrofuran in the dark for 18 h. Finally, the α -bromoacryloylamido derivatives **5a-g** were prepared by the condensation of α -bromoacrylic anhydride (generated by the treatment of α -bromoacrylic acid with DCC in acetonitrile) with 3-aminobenzoheterocycles **2a-g**, respectively.

2.2. Chemistry. Experimental section

¹H 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 ¹H NMR spectra in agreement with the assigned structures. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise indicated. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F₂₅₄ Merck plates) and visualized with aqueous KMnO₄. Flash chromatography was performed using 230–400 mesh silica gel and the indicated solvent system. Organic solutions were dried over anhydrous Na₂SO₄. Calcium chloride was used in the distillation of DMF, and the distilled solvent was stored over molecular sieves (3 Å).

2.2.1. General Procedure (A) for the Synthesis of Compounds 3a, 3e, 3i, 3m, 3q, 3u and 3y—To a solution of **2a-g** (1 mmol) and pyridine (3 mmol, 242 μ L) in dry dichloromethane (5 mL), acetyl chloride (3 mmol, 212 μ L) was added at 0 °C. The reaction mixture was stirred for 2 h at room temperature, diluted with dichloromethane (5 mL), washed with water (4 mL), dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified by flash chromatography.

2.2.1.1. N-[2-(3,4,5-Trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3a): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3a** as a yellow solid (91% yield); mp 173–174 °C. ¹H-NMR (CDCl₃) δ: 2.36 (s, 3H), 3.97 (s, 3H), 3.98 (s, 6H), 7.32 (m, 1H), 7.44 (m, 2H), 7.52 (s, 2H), 8.53 (d, *J*=8.0 Hz, 1H), 10.7 (bs, 1H).

2.2.1.2. N-[5-Methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3e): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3e** as a yellow solid (89% yield); mp 150–151 °C. ¹H-NMR (CDCl₃) δ: 2.36 (s, 3H), 3.90 (s, 3H), 3.96 (s, 3H), 3.97 (s, 6H), 7.16 (d, *J*=8.8 Hz, 1H), 7.36 (d, *J*=8.8 Hz, 1H), 7.55 (s, 2H), 7.98 (s, 1H), 10.7 (s, 1H).

2.2.1.3. N-[6-Methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3i): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3i** as a yellow solid (90% yield); mp 172–173 °C. ¹H-NMR (CDCl₃) δ: 2.17 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 3.97 (s, 6H), 6.88 (s, 1H), 6.90 (d, *J*=9.2 Hz, 1H), 7.53 (s, 2H), 8.47 (d, *J*=9.2 Hz, 1H), 10.9 (s, 1H).

2.2.1.4. N-[7-Methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3m): Following general procedure (A) the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **3m** as a yellow solid (82% yield); mp 188–190 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 2.18 (s, 3H), 3.98 (s, 6H), 3.99 (s, 3H), 6.92 (d, *J*=8.0 Hz, 1H), 7.22 (m, 1H), 7.72 (s, 2H), 8.06 (d, *J*=8.0 Hz, 1H), 11.3 (s, 1H).

2.2.1.5. N-[2-(3,4,5-Trimethoxybenzoyl)-1-benzothien-3-yl] acetamide (3q): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3q** as a yellow solid (72% yield); mp 170–171 °C. ¹H-NMR (CDCl₃) δ: 2.33 (s, 3H), 3.93 (s, 3H), 3.96 (s, 6H), 7.26 (s, 2H), 7.44 (d, *J*=7.2 Hz, 1H), 7.51 (d, *J*=7.2 Hz, 1H), 7.76 (d, *J*=8.0 Hz, 1H), 8.09 (d, *J*=8.0 Hz, 1H), 10.2 (bs, 1H).

2.2.1.6. N-[6-Methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3u): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3u** as a yellow solid (78% yield); mp 212–213 °C. ¹H-NMR (CDCl₃) δ: 1.58 (s, 3H), 2.17 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 7.23 (d, *J*=8.4 Hz, 1H), 7.26 (s, 2H), 7.55 (s, 1H), 8.00 (d, *J*=8.4 Hz, 1H), 10.4 (s, 1H).

2.2.1.7. N-[7-Methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3y): Following general procedure (A), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3y** as a yellow solid (67% yield); mp 137–138 °C. ¹H-NMR (CDCl₃) δ: 1.58 (s, 3H), 2.32 (s, 3H), 3.93 (s, 6H), 3.96 (s, 3H), 7.29 (s, 2H), 7.38 (m, 2H), 7.92 (d, *J*=7.2 Hz, 1H), 10.2 (s, 1H).

2.2.2. General Procedure (B) for the Synthesis of Compounds 3b, 3f, 3j, 3n, 3r, 3v and 3z—To a solution of **2a-g** (1 mmol) and pyridine (3 mmol, 242 μL) in dry dichloromethane (5 mL), chloroacetyl chloride (3 mmol, 239 μL) was added at 0 °C. The reaction mixture was stirred for 1 h at room temperature, diluted with dichloromethane (10 mL), washed with water (5 mL), dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified by flash chromatography.

2.2.2.1. 2-Chloro-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3b): Following general procedure (B), the crude residue purified by flash chromatography using

ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **3b** as a green solid (86% yield); mp 187–190 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 4.33 (s, 2H), 7.33 (s, 1H), 7.47 (m, 2H), 7.58 (s, 2H), 8.53 (d, *J*=8.4 Hz, 1H), 11.6 (bs, 1H).

2.1.2.2. 2-Chloro-N-[5-methoxy-2-(3,4,5-trimethoxy-benzoyl)-1-benzofuran-3-yl]acetamide (3f): Following general procedure (B), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3f** as a yellow solid (95% yield); mp 184–186 °C. ¹H-NMR (CDCl₃) δ: 3.90 (s, 3H), 3.97 (s, 3H), 3.98 (s, 6H), 4.32 (s, 2H), 7.17 (d, *J*=9.2 Hz, 1H), 7.38 (d, *J*=9.2 Hz, 1H), 7.57 (s, 2H), 7.95 (s, 1H), 11.6 (s, 1H).

2.1.2.3. 2-Chloro-N-[6-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3j): Following general procedure (B) the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3j** as a green solid (>95% yield); mp 123–124 °C. ¹H-NMR (CDCl₃) δ: 3.91 (s, 3H), 3.97 (s, 9H), 4.31 (s, 2H), 6.91 (s, 1H), 6.95 (d, *J*=9.2 Hz, 1H), 7.55 (s, 2H), 8.47 (d, *J*=9.2 Hz, 1H), 11.7 (s, 1H).

2.2.2.4. 2-Chloro-N-[7-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3n): Following general procedure (B) the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **3n** as a white solid (>95% yield); mp 210–212 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 3.99 (s, 3H), 4.31 (s, 2H), 7.00 (d, *J*=8.0 Hz, 1H), 7.24 (m, 1H), 7.70 (s, 2H), 8.05 (d, *J*=8.0 Hz, 1H), 11.5 (s, 1H).

2.2.2.5. 2-Chloro-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3r): Following general procedure (B), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3r** as a yellow solid (95% yield); mp 151–153 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 6H), 3.96 (s, 3H), 4.30 (s, 2H), 7.27 (s, 2H), 7.48 (d, *J*=7.2 Hz, 1H), 7.53 (d, *J*=7.2 Hz, 1H), 7.81 (d, *J*= 8.0 Hz, 1H), 8.03 (d, *J*= 8.0 Hz, 1H), 10.9 (bs, 1H).

2.2.2.6. 2-Chloro-N-[6-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3v): Following general procedure (B), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3v** as a yellow solid (82% yield); mp 154–155 °C. ¹H-NMR (CDCl₃) δ: 2.50 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 4.30 (s, 2H), 7.26 (s, 2H), 7.29 (d, *J*=8.8 Hz, 1H), 7.59 (s, 1H), 7.96 (d, *J*=8.8 Hz, 1H), 11.0 (s, 1H).

2.2.2.7. 2-Chloro-N-[7-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3z): Following general procedure (B), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3z** as a yellow solid (62% yield); mp 185–186 °C. ¹H-NMR (CDCl₃) δ: 2.54 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 4.28 (s, 2H), 7.29 (s, 2H), 7.36 (m, 2H), 7.86 (d, *J*=7.2 Hz, 1H), 10.8 (s, 1H).

2.2.3. General Procedure (C) for the Preparation of 3c, 3g, 3k, 3o, 3s, 3w and 3aa—To a solution of **2a-g** (1 mmol) and pyridine (3 mmol, 242 μL) in dry dichloromethane (5 mL), bromoacetyl chloride (3 mmol, 250 μL) was added at 0 °C. After 3 h at the same temperature, the reaction mixture was diluted with dichloromethane (5 mL), washed with water (5 mL), dried over Na₂SO₄ and concentrated in vacuo. The crude residue was purified by flash chromatography.

2.2.3.1. 2-Bromo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3c):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3c** as a green solid (78% yield); mp 193–195 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 4.14 (s, 2H), 7.31 (s, 1H), 7.49 (m, 2H), 7.59 (s, 2H), 8.49 (d, *J*=8.0 Hz, 1H), 11.4 (bs, 1H).

2.2.3.2. 2-Bromo-N-[5-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3g):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3g** as a yellow solid (95% yield); mp 178–180 °C. ¹H-NMR (CDCl₃) δ: 3.90 (s, 3H), 3.92 (s, 3H), 3.97 (s, 6H), 4.14 (s, 2H), 7.17 (d, *J*=8.8 Hz, 1H), 7.39 (d, *J*=8.8 Hz, 1H), 7.56 (s, 2H), 7.92 (s, 1H), 11.4 (s, 1H).

2.2.3.3. 2-Bromo-N-[6-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3k):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3k** as a green solid (78% yield), mp 99–100 °C. ¹H-NMR (CDCl₃) δ: 3.89 (s, 3H), 3.92 (s, 6H), 3.97 (s, 3H), 4.13 (s, 2H), 6.91 (s, 1H), 6.94 (d, *J*=9.2 Hz, 1H), 7.54 (s, 2H), 8.45 (d, *J*=9.2 Hz, 1H), 11.6 (s, 1H).

2.2.3.4. 2-Bromo-N-[7-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3o):

Following general procedure (C) the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **3o** as a white solid (72%, yield); mp 215–217 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 3.99 (s, 3H), 4.13 (s, 2H), 7.00 (d, *J*=7.6 Hz, 1H), 7.23 (m, 1H), 7.69 (s, 2H), 8.04 (d, *J*=8.4 Hz, 1H), 11.3 (s, 1H).

2.2.3.5. 2-Bromo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3s):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3s** as a green solid (76% yield); mp 155–157 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 6H), 3.96 (s, 3H), 4.11 (s, 2H), 7.26 (s, 2H), 7.48 (d, *J*=7.2 Hz, 1H), 7.53 (d, *J*=7.2 Hz, 1H), 7.80 (d, *J*= 8.0 Hz, 1H), 8.02 (d, *J*= 8.0 Hz, 1H), 10.8 (bs, 1H).

2.2.3.6. 2-Bromo-N-[6-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3w):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3w** as a brown solid (82% yield); mp 160–163 °C. ¹H-NMR (CDCl₃) δ: 2.50 (s, 3H), 3.92 (s, 6H), 3.95 (s, 3H), 4.11 (s, 2H), 7.22 (d, *J*=8.6 Hz, 1H), 7.26 (s, 2H), 7.58 (s, 1H), 7.96 (d, *J*=8.6 Hz, 1H), 10.9 (s, 1H).

2.2.3.7. 2-Bromo-N-[7-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3aa):

Following general procedure (C), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3aa** as a yellow solid (52% yield); mp 169–170 °C. ¹H-NMR (CDCl₃) δ: 2.53 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 4.10 (s, 2H), 7.25 (s, 2H), 7.38 (m, 2H), 7.84 (d, *J*=7.2 Hz, 1H), 10.6 (s, 1H).

2.2.4. General Procedure (D) for the Synthesis of 3d, 3h, 3l, 3p, 3t, 3x, 3ab—

A mixture of bromoacetamido derivatives **3c**, **3g**, **3k**, **3o**, **3s**, **3w** and **3aa** (1 mmol) and NaI (10 mmol, 1.5 g) in *N,N*-dimethylacetamide (5 mL) was stirred at room temperature for 18 h. *N,N*-dimethylacetamide was evaporated under reduced pressure, followed by addition of dichloromethane (15 mL) and a solution of Na₂S₂O₃ (10%, 5 mL). The organic layer was

washed with water (5 mL), brine (5 mL) and dried over Na₂SO₄. After removal of the solvent under reduced pressure, the crude residue was purified by flash chromatography.

2.2.4.1. 2-Iodo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3d):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3d** as a brown solid (83% yield); mp 212–213 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 4.01 (s, 2H), 7.29 (s, 1H), 7.52 (m, 2H), 7.58 (s, 2H), 8.46 (d, J=8.2 Hz, 1H), 11.6 (bs, 1H).

2.2.4.2. 2-Iodo-N-[5-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3h):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3h** as a white solid (77% yield); mp 184–186 °C. ¹H-NMR (CDCl₃) δ: 3.90 (s, 3H), 3.97 (s, 3H), 3.98 (s, 6H), 4.00 (s, 2H), 7.16 (d, J=8.8 Hz, 1H), 7.37 (d, J=8.8 Hz, 1H), 7.56 (s, 2H), 7.93 (s, 1H), 11.1 (s, 1H).

2.2.4.3. 2-Iodo-N-[6-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3l):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **3l** as a yellow solid (58% yield) after recrystallization from petroleum ether; mp 140–141 °C. ¹H-NMR (CDCl₃) δ: 3.72 (s, 2H), 3.90 (s, 3H), 3.97 (s, 6H), 3.98 (s, 3H), 6.89 (s, 1H), 6.92 (d, J=8.8 Hz, 1H), 7.54 (s, 2H), 8.45 (d, J=8.8 Hz, 1H), 11.5 (s, 1H).

2.2.4.4. 2-Iodo-N-[7-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acetamide (3p):

Following general procedure (D) the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **3p** as a yellow solid (52% yield); mp 211–213 °C. ¹H-NMR (CDCl₃) δ: 3.98 (s, 3H), 3.99 (s, 9H), 4.00 (s, 3H), 7.00 (d, J=8.0 Hz, 1H), 7.24 (m, 1H), 7.69 (s, 2H), 8.04 (d, J=8.2 Hz, 1H), 11.0 (s, 1H).

2.2.4.5. 2-Iodo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3t):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 2:8 (v:v) as eluent furnished **3t** as a white solid (83% yield); mp 179–180 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 6H), 3.96 (s, 3H), 3.97 (s, 2H), 7.26 (s, 2H), 7.49 (d, J=7.2 Hz, 1H), 7.54 (d, J=7.2 Hz, 1H), 7.78 (d, J= 7.8 Hz, 1H), 8.02 (d, J= 7.8 Hz, 1H), 10.5 (bs, 1H).

2.2.4.6. 2-Iodo-N-[6-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3x):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3x** as a brown solid (97% yield); mp 145–147 °C. ¹H-NMR (CDCl₃) δ: 2.49 (s, 3H), 3.93 (s, 6H), 3.95 (s, 3H), 3.97 (s, 2H), 7.21 (d, J=7.8 Hz, 1H), 7.26 (s, 2H), 7.47 (s, 1H), 7.88 (d, J=7.8 Hz, 1H), 10.7 (s, 1H).

2.2.4.7. 2-Iodo-N-[7-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acetamide (3ab):

Following general procedure (D), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **3ab** as a yellow solid (84% yield); mp 186–188 °C. ¹H-NMR (CDCl₃) δ: 2.54 (s, 3H), 3.93 (s, 6H), 3.96 (s, 5H), 7.22 (s, 2H), 7.36 (m, 2H), 7.89 (d, J=7.8 Hz, 1H), 11.0 (s, 1H).

2.2.5. General Procedure (E) for the synthesis of compounds 4a-d—To a solution of bromoacetamido derivative (1 mmol) in tetrahydrofuran (10 mL), silver nitrate (510 mg, 3 mmol, 3 equiv.) was added. The mixture was stirred at reflux in the dark for 18 h. The precipitate was removed by filtration and the solvent evaporated under vacuum. The residue was extracted in a mixture of dichloromethane (15 mL) and water (5 mL). The

organic phase was washed with brine, dried (Na₂SO₄) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel..

2.2.5.1. 2-Oxo-2-[[2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]amino]ethyl nitrate (4a): Following general procedure (E), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **4a** as a brown solid (54% yield); mp 178–180 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 5.14 (s, 2H), 7.37 (m, 1H), 7.51 (m, 2H), 7.58 (s, 2H), 8.51 (d, J=8.8 Hz, 1H), 11.5 (bs, 1H).

2.2.5.2. 2-Oxo-2-[[2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]amino]ethyl nitrate (4b): Following general procedure (E), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **4b** as a white solid (63% yield); mp 184–185 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 6H), 3.95 (s, 3H), 5.14 (s, 2H), 7.26 (s, 2H), 7.54 (m, 2H), 7.78 (d, J= 7.2 Hz, 1H), 8.05 (d, J= 7.2 Hz, 1H), 10.8 (bs, 1H).

2.2.5.3. 2-[[6-Methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]amino]-2-oxoethyl nitrate (4c): Following general procedure (E), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **4c** as a white solid (55% yield); mp 182–183 °C. ¹H-NMR (CDCl₃) δ: 2.49 (s, 3H), 3.93 (s, 6H), 3.95 (s, 3H), 5.12 (s, 2H), 7.201 (d, J=8.6 Hz, 1H), 7.24 (s, 2H), 7.58 (s, 1H), 8.00 (d, J=8.6 Hz, 1H), 10.9 (s, 1H).

2.2.5.4. 2-[[7-Methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]amino]-2-oxoethyl nitrate (4d): Following general procedure (E), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **4d** as a yellow oil (64% yield). ¹H-NMR (CDCl₃) δ: 2.53 (s, 3H), 3.93 (s, 6H), 3.96 (s, 3H), 5.14 (s, 2H), 7.23 (s, 2H), 7.39 (m, 2H), 7.90 (d, J=7.8 Hz, 1H), 10.7 (s, 1H).

2.2.6. General Procedure (F) for the Synthesis of Compounds 5a-g—To an ice-cooled mixture of α-bromoacrylic acid (306 mg, 2 mol) in acetonitrile (4 mL) was slowly added a solution of DCC (210 mg, 1 mmol) in acetonitrile (4 mL). The mixture was stirred for 3 h at room temperature and filtered, and the filtrate was added dropwise to a solution of amino derivatives **2a-g** (1 mmol) in acetonitrile (5 mL). The reaction was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was dissolved with a mixture of DCM (15 mL) and water (5 mL), and the organic phase was washed with brine (5 mL), dried over Na₂SO₄ and evaporated to dryness *in vacuo*. The resulting crude residue was purified by flash chromatography.

2.2.6.1. 2-Bromo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acrylamide (5a): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **5a** as a white solid (56% yield); mp 174–176 °C. ¹H-NMR (CDCl₃) δ: 3.97 (s, 3H), 3.98 (s, 6H), 6.27 (s, 1H), 7.22 (s, 1H), 7.28 (s, 2H), 7.34 (m, 1H), 7.51 (m, 2H), 8.56 (d, J=7.2 Hz, 1H), 11.7 (bs, 1H).

2.2.6.2. 2-Bromo-N-[5-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acrylamide (5b): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **5b** as a white solid (78% yield); mp 134–135 °C. ¹H-NMR (CDCl₃) δ: 3.94 (s, 3H), 3.95 (s, 3H), 3.97 (s, 6H), 6.39 (s, 1H), 7.08 (s, 1H), 7.19 (d, J=8.0 Hz, 1H), 7.39 (d, J=8.8 Hz, 1H), 7.57 (s, 2H), 8.14 (s, 1H), 110.7 (s, 1H).

2.2.6.3. 2-Bromo-N-[6-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acrylamide (5c): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **5c** as a yellow oil (78% yield). ¹H-NMR (CDCl₃) δ: 3.90 (s, 3H), 3.93 (s, 3H), 3.96 (s, 6H), 6.42 (s, 1H), 6.89 (s, 1H), 6.92 (d, *J*=9.2 Hz, 1H), 7.02 (s, 1H), 7.50 (s, 2H), 8.44 (d, *J*=9.2 Hz, 1H), 10.7 (s, 1H).

2.2.6.4. 2-Bromo-N-[7-methoxy-2-(3,4,5-trimethoxybenzoyl)-1-benzofuran-3-yl]acrylamide (5d): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 1:1 (v:v) as eluent furnished **5d** as a white solid (63% yield), mp 183–184 °C. ¹H-NMR (CDCl₃) δ: 3.92 (s, 3H), 3.96 (s, 3H), 3.99 (s, 6H), 6.38 (s, 1H), 6.84 (d, *J*=7.2 Hz, 1H), 7.09 (s, 1H), 7.24 (s, 2H), 7.38 (m, 1H), 8.32 (d, *J*=7.2 Hz, 1H), 11.7 (s, 1H).

2.2.6.5. 2-Bromo-N-[2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acrylamide (5e): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **5e** as a white solid (58% yield); mp 183–185 °C. ¹H-NMR (CDCl₃) δ: 3.93 (s, 3H), 3.96 (s, 6H), 6.24 (s, 1H), 6.38 (s, 1H), 7.26 (s, 2H), 7.48 (d, *J*=8.0 Hz, 1H), 7.53 (d, *J*=8.0 Hz, 1H), 7.80 (d, *J*=8.0 Hz, 1H), 8.04 (d, *J*=8.0 Hz, 1H), 11.1 (bs, 1H).

2.2.6.6. 2-Bromo-N-[6-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acrylamide (5f): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 4:6 (v:v) as eluent furnished **5f** as a white solid (76% yield); mp 181–183 °C. ¹H-NMR (CDCl₃) δ: 2.51 (s, 3H), 3.92 (s, 6H), 3.96 (s, 3H), 6.23 (s, 1H), 7.09 (s, 2H), 7.17 (s, 1H), 7.26 (d, *J*=8.4 Hz, 1H), 7.59 (s, 1H), 7.96 (d, *J*=8.4 Hz, 1H), 11.2 (s, 1H).

2.2.6.7. 2-Bromo-N-[7-methyl-2-(3,4,5-trimethoxybenzoyl)-1-benzothien-3-yl]acrylamide (5g): Following general procedure (F), the crude residue purified by flash chromatography using ethyl acetate: petroleum ether 3:7 (v:v) as eluent furnished **5g** as a yellow oil (56% yield). ¹H-NMR (CDCl₃) δ: 2.53 (s, 3H), 3.93 (s, 6H), 3.96 (s, 3H), 6.68 (s, 1H), 7.12 (s, 1H), 7.30 (s, 2H), 7.36 (m, 2H), 7.87 (d, *J*=7.6 Hz, 1H), 10.5 (s, 1H).

2.3. Biological Assays

2.3.1. Cell Proliferation Analysis—The murine lymphocytic L1210 leukemia and the human chronic myelogenous K562 cell lines were obtained from the American Type Culture Collection (ATCC). All the tested compounds were dissolved in DMSO at 1 mg/mL immediately before the use and stored as stock solutions at –20°C. Further dilutions were made in culture medium before addition to the cells. In all experiments, the final concentration of DMSO did not exceed 0.3 % v/v, a concentration which was non-toxic to the cells. Both cell lines were cultured in RPMI 1640 medium (GIBCO) supplemented with 10% FCS (Flow, Irvine, U.K.), 2 mM L-glutamine (GIBCO), 10 mM β-mercaptoethanol, 100 U/mL penicillin and 100 µg/mL streptomycin in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Cells were maintained at a density of 1×10⁶ cells/mL. To determine the effects of the studied compounds on *in vitro* cell growth, L1210 and K562 cells (1×10⁴/well) were exposed to increasing concentrations of drugs in 96-well plates and the value of cell number/ml was determined after 24h of cell culture at 37°C using a ZF Coulter Counter (Beckman Coulter Electronics, Hialeah, Fla., USA). Results were expressed as IC₅₀ (dose causing 50% inhibition of cell growth in treated cultures relative to untreated controls). All experiments were repeated at least twice. For each drug concentration, duplicate cultures were used.

2.3.2. Effects on Tubulin Polymerization and Colchicine Binding—To evaluate the effect of the compounds on tubulin assembly *in vitro*, varying concentrations were preincubated with 10 μ M tubulin in 0.8 M monosodium glutamate buffer at 30 °C and then cooled to 0 °C. After addition of 0.4 mM 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 IC₅₀ was defined as the compound concentration that inhibited the extent of assembly by 50% after 20 min at 30 °C. In the colchicine binding assay, tubulin at 1.0 μ M was incubated with 5.0 μ M [³H]colchicine and tested compounds at 1.0 μ M for 10 min at 37 °C.

2.3.3. Cell Cycle Analysis—The effects of the most active compounds of the series on cell cycle distribution were studied on K562 cells (chronic myeloblastic leukemia) by flow cytometric analysis after staining with propidium iodide. K562 cells were cultured in RPMI 1640 (Gibco Grand Island, NY, USA) containing 10% FCS (Gibco), 100 U/ml penicillin (Gibco), 100 μ g/ml streptomycin (Gibco), and 2 mM l-glutamine (Sigma Chemical Co., St. Louis, MO) in a 5% CO₂ atmosphere at 37 °C.

Cells were exposed 24 h to each compound used at a concentration corresponding to the IC₅₀ determined after a 24 h incubation. After treatment, the cells were washed once in ice-cold PBS and resuspended at 1 $\times$ 10⁶ per mL in a hypotonic fluorochrome solution containing propidium iodide (Sigma) at 50 μ g/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 using MultiCycle for Windows (Phoenix Flow Systems, San Diego, CA).

3. RESULTS AND DISCUSSION

3.1. *In Vitro* Antiproliferative Activities

Table 1 summarizes the effects of compounds **2a-g**, **3a-ab**, **4a-d** and **5a-g** on the growth of murine L1210 and human chronic myelogenous K562 leukemia cells. The L1210 antiproliferative assay is valued as a preliminary indication of potential activity of anticancer drugs [19]. The K562 cell line was used for initial compound screening because of its rapid proliferation and high sensitivity to standard anticancer agents and in order to determine whether these compounds had activity against human transformed cells [19,20]. Out of the synthesized molecules, twenty-one exhibited good activity with IC₅₀ values at submicromolar concentrations, including seven compounds with IC₅₀ values lower than 100 nM. The antiproliferative data showed that the position of the methoxy substituent on the phenyl moiety of the benzo[b]furan ring greatly affected activity (**3i-l** vs. **3e-h** and **3m-p**), while in the series of benzo[b]thiophene derivatives, compounds bearing methyl substituents at the C-6 or C-7 position had similar activity (**3u-x** vs. **3y-ab**). Comparing the molecules which possessed the same substituent at the 3-position on the benzoheterocyclic ring, the benzo[b]furan derivatives are much less active than their benzo[b]thiophene counterparts.

In the series of unsubstituted benzo[b]furan derivatives **3b-d**, the iodine derivative **3d** was 6-fold more potent than the bromine **3c** and from 20- to 60-fold more active than the chlorine **3b**. Thus, compounds **3b-d** are ranked according to their activity as iodoacetyl>bromoacetyl>chloroacetyl.

In the series of methoxy substituted benzo[b]furan acetamido and haloacetamido derivatives **3e-p**, pronounced inhibition of cell growth of this class of compounds required a methoxy group at the C-6 position (compounds **3i-l**). In contrast, only limited activity was observed

with the substituent placed at the C-5 or C-7 positions (derivatives **3e-h** and **3m-p**, respectively).

Comparing the benzo[b]thiophene derivatives **3q-ab**, compounds **3u-x** and **3y-ab** bearing a methyl substituent at either the C-6 or C-7 position, respectively, enhanced activity was observed relative to the unsubstituted derivatives **3q-t**.

In the series of nitrooxyacetamido compounds **4a-d**, **4d** was the most active, and their activity was ranked in the order: **4d**>**4c**>**4b**>>**4a**. Starting from the unsubstituted derivative **4b**, the introduction of the methyl group at the C-6 position of the benzo[b]thiophene nucleus (compound **4c**) increased the potency 20-fold against K562 cells, while **4b** and **4c** exhibited similar activity against L1210 cells. The C-5 and C-7 methyl benzo[b]thiophene derivatives **4c** and **4d**, respectively, exhibited comparable activity against K562 cells, while **4d** was about 5-fold more potent than **4c** against the L1210 cells.

For the α -bromoacryloylamido derivatives **5a-g**, the un-substituted benzo[b]furan derivative **5a** was 60-fold more active than the corresponding amino counterpart **2a** against L1210 cells, demonstrating that the presence of an α -bromoacryloyl moiety significantly enhanced antiproliferative activity. In the series of methoxy substituted analogues **2b-d**, the introduction of the α -bromoacryloyl moiety was detrimental for activity. The bioisosteric replacement of benzo[b]furan **5a** with benzo[b]thiophene **5e** greatly increased activity, with IC₅₀ values of 0.58 and 0.0077 μ M versus 5.1 and 0.53 μ M against K562 and L1210 cells, respectively. The introduction of a methyl group at the C-6 or C7-position, to furnish **5f** and **5g**, respectively, had a minimal effect on activity.

3.2. Inhibition of Tubulin Polymerization and Colchicine Binding

Previous work had shown that compounds **2c**, **2f** and **2g** most likely derived their potent antiproliferative activity from a strong interaction at the colchicine site of tubulin [12]. We therefore continue to analyze the compounds with especially potent antiproliferative activity for their effects on tubulin assembly and the binding of [³H]colchicine to tubulin. In Table 2 we present data with the 6-methoxy benzo[b]furan derivatives **3i-l** [methodology in references 21,22].

The order of inhibitory action on tubulin assembly was **3i**>**3l**>**3j**>**3k**, which was consistent with the results of the antiproliferative assays. The most potent compound in this series was compound **3i**, with an IC₅₀ value of 1.6 μ M. This is in agreement with **3i** being the compound with the greatest antiproliferative activity. In the colchicine binding studies, compounds **3i-l** inhibited the binding of [³H]colchicine to tubulin, with 47–54% inhibition occurring with these agents at 1 μ M and colchicine at 5 μ M. This represents strong inhibition of colchicine binding, almost as great as that observed with the particularly potent combretastatin A-4 at 1 μ M (generally 85–90%).

3.3. Analysis of Cell Cycle

Because molecules exhibiting effects on tubulin assembly should cause alteration of cell cycle parameters with preferential G2-M blockade, we examined cell cycle effects of derivatives **3i-l** using flow cytometric analysis on K562 cells. The cells were cultured for 48 h in the presence of each compound at the IC₅₀ value. The majority of control cells were in S phase (54%), with 33% of the cells in the G0-G1 phase and 13% in the G2/M phase [23–25]. Analysis of sub-G0-G1 cells (apoptotic peak), G0-G1, S, and G2-M peaks following compound treatment revealed that each compound caused somewhat different effects on cell cycle distribution (Table 3). While all four compounds caused an increase in the proportion of cells in the G2-M peak, compound **3j** also caused cells to accumulate in late S phase (data not shown). Compound **3l** caused an increase in the percentage of cells in S phase, and it

yielded the largest increase in apoptotic cells, as shown by the largest increase in the sub G0-G1 peak.

CONCLUSIONS

The structure-activity relationship (SAR) study for the anticancer effect of the substituents at the amino group in the 3. position of the benzo[*b*]furan or benzo[*b*]thiophene ring was carried out by the introduction of different acyl substituents such as acetyl, chloroacetyl, bromoacetyl, iodoacetyl, α -bromoacryloyl and nitrooxyacetyl. The antiproliferative activity of unsubstituted benzo[*b*]furan derivatives **3b-d** appeared to be ranked in an order of the nature of halogens: I>Br>Cl. For the derivatives **3e-p**, the results indicated that the inhibition of cell growth was strongly dependent on the position of the methoxy moiety. A comparison of substituent effect revealed that the C6-methoxy compounds **3i-l** exceed that of their C-5 and C-7 methoxy counterparts **3e-h** and **3m-p**, respectively, by 2-orders of magnitude. It is noteworthy that the antiproliferative effects of **3i-l** were more pronounced against murine human T-lymphoblastoid (Molt/4 and CEM) as compared with mammary carcinoma (FM3A) cells [13]. In the series of benzo[*b*]thiophene derivatives, moving the methyl group from C6- to C7-position, only a marginal improvement of antiproliferative activity occurred. The study has shown that this class of compounds exerts antiproliferative effects through inhibition of tubulin polymerization and cell cycle arrest in G2-M phase.

A group of nitric oxide donor derivatives **4a-d** were designed to investigate the concept whether the release of cytotoxic NO may enhance the antiproliferative efficacy of 2-(3', 4', 5'-trimethoxybenzoyl)-benzo[*b*]furan and benzo[*b*]thiophene moieties. The benzo[*b*]thiophene analogues **4b-d** exhibit comparable antiproliferative activity to chloroacetyl counterparts **3r**, **3v** and **3z**, respectively, and deserved further evaluation for their effect on the proliferation of non-leukemic cancer cell lines.

The novel series of α -bromoacryloyl benzoheterocycles **5a-g** were found to inhibit the growth murine leukemia L1210 cells, with compounds **5e-g** that showed strong antiproliferative activities, with IC₅₀ values <1 μ M on human leukemia K562 cells.

The strong antiproliferative activity of 6-methoxy benzo[*b*]furan derivatives **3i-l** along with the 6- and 7-methyl benzo[*b*]thiophene analogues **3u-x** and **3y-ab** justify further exploration of their potential antitumor activity and to prepare additional analogs in hope of further elucidating important SAR features and identifying other active congeners. Further studies to clarify additional details of the molecular mechanism of action of these compounds and the selectivity to inhibit the growth of additional human acute myeloid leukemia cells are underway.

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Scheme 1.

Synthesis of compounds **3a-ab**, **4a-d** and **5a-g**.

Reagents and conditions. **a**: CH₃COCl, Py, CH₂Cl₂, rt for the synthesis of **3a**, **3e**, **3i**, **3m**, **3q**, **3u** and **3y**; **b**: ClCH₂COCl, Py, CH₂Cl₂, rt for the preparation of **3b**, **3f**, **3j**, **3n**, **3r**, **3v** and **3z**; **c**: BrCH₂COBr, Py, CH₂Cl₂, rt for the preparation of **3c**, **3g**, **3k**, **3o**, **3s**, **3w** and **3aa**; **d**: NaI, CH₃CON(CH₃)₂, rt; **e**: AgNO₃, THF, reflux; **f**: α-bromoacrylic acid, DCC, CH₃CN, rt.

X=F, Cl, Br or I
R=CH₃, halogen, OCH₃, NO₂, CO₂CH₃, OH

2a, A=O, R=H
2b, A=O, R=5-OCH₃
2c, A=O, R=6-OCH₃
2d, A=O, R=7-OCH₃
2e, A=S, R=H
2f, A=S, R=6-CH₃
2g, A=S, R=7-CH₃

A=O or S
R=H, Me or OMe
X=H, Cl, Br or I

4a, A=O, R=H
4b, A=S, R=H
4c, A=S, R=6-CH₃
4d, A=S, R=7-CH₃

5a, A=O, R=H
5b, A=O, R=5-OCH₃
5c, A=O, R=6-OCH₃
5d, A=O, R=7-OCH₃
5e, A=S, R=H
5f, A=S, R=6-CH₃
5g, A=S, R=7-CH₃

Chart 1.
General chemical structures of compounds **1–5**.

Table 1

In Vitro Inhibitory Effects of Compounds 2a-g, 3a-ab, 4a-d and 5a-g on Proliferation of Human K562 and Murine L1210 Leukemia Cell Lines

Compd	IC ₅₀ (μM)		Compd	IC ₅₀ (μM)	
	K562	L1210		K562	L1210
2a	8.29 ± 1.48	32.4 ± 0.89	3q	0.63 ± 0.04	1.89 ± 1.22
2b	7.20 ± 1.12	8.38 ± 1.45	3r	0.60 ± 0.02	0.46 ± 0.49
2c	0.008 ± 0.00	0.042 ± 0.01	3s	0.61 ± 0.04	0.5 ± 0.01
2d	0.725 ± 0.11	6.39 ± 1.2	3t	0.76 ± 0.02	0.58 ± 0.09
2e	0.53 ± 0.01	0.66 ± 0.06	3u	0.70 ± 0.01	0.58 ± 0.01
2f	0.006 ± 0.00	0.009 ± 0.00	3v	0.08 ± 0.00	0.059 ± 0.00
2g	0.03 ± 0.01	0.062 ± 0.00	3w	0.5 ± 0.08	0.24 ± 0.18
3a	>100	>100	3x	0.61 ± 0.01	0.44 ± 0.07
3b	45.8 ± 1.5	51.8 ± 15.1	3y	0.032 ± 0.00	0.053 ± 0.00
3c	12.8 ± 0.51	4.56 ± 0.64	3z	0.04 ± 0.02	0.05 ± 0.00
3d	2.21 ± 0.09	0.81 ± 0.00	3aa	0.49 ± 0.08	0.25 ± 0.18
3e	58.1 ± 1.3	64.7 ± 2.0	3ab	0.55 ± 0.01	0.28 ± 0.08
3f	60.9 ± 2.4	49.0 ± 1.7	4a	31.4 ± 1.0	33.4 ± 2.8
3g	53.4 ± 2.6	53.0 ± 3.0	4b	0.55 ± 0.05	0.31 ± 0.02
3h	49.23 ± 1.8	0.52 ± 0.03	4c	0.03 ± 0.02	0.28 ± 0.03
3i	0.027 ± 0.01	0.060 ± 0.01	4d	0.05 ± 0.01	0.055 ± 0.01
3j	0.030 ± 0.02	0.056 ± 0.01	5a	5.1 ± 0.07	0.53 ± 0.00
3k	0.180 ± 0.03	0.72 ± 0.12	5b	>100	>100
3l	0.064 ± 0.02	0.57 ± 0.11	5c	25.9 ± 2.3	5.02 ± 0.89
3m	48.1 ± 2.6	52.7 ± 2.3	5d	55.9 ± 4.6	26.8 ± 3.2
3n	82.74 ± 5.6	62.68 ± 4.4	5e	0.58 ± 0.03	0.077 ± 0.01
3o	>100	>100	5f	0.94 ± 0.06	0.059 ± 0.00
3p	6.96 ± 1.12	42.84 ± 3.77	5g	0.49 ± 0.03	0.06 ± 0.01

^aIC₅₀= 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.

Table 2

Inhibition of Tubulin Polymerization and Colchicine Binding by Compounds 3i-l

Compound	Tubulin assembly ^a IC ₅₀ ±SD (μM)	Colchicine binding ^b % ±SD
3i	1.6±0.0	51±7
3j	2.0±0.1	47±5
3k	2.3±0.5	49±2
3l	1.8±0.1	54±1

^aInhibition of tubulin polymerization. Tubulin was at 10 μM.

^bInhibition of [³H]colchicine binding. Tubulin, colchicine and tested compound were at 1, 5 and 1 μM, respectively. nd: not determined.

Table 3
Effects of Compounds 3i-l on Cell Cycle Distribution of Treated K562 Cells

Compd	IC ₅₀ (nM)	Cell cycle percentage			
		Sub-G1 ^a	G0/G1 ^b	S ^b	G2/M ^b
Control		2±0	33±2	54±5	13±2
3i	85	12±3	27±5	36±8	37±6
3j	400	13±2	23±4	44±7	33±5
3k	212	15±2	6±1	27±4	67±8
3l	115	33±4	23±3	56±4	21±3

Data are expressed as the mean ± SE from the dose-response curves of at least three independent experiments.

^aPercentage of the cell population with hypodiploid DNA content peak (apoptotic cells).

^bThe percentage of cells in each phase of the cell cycle was calculated using living cells.