Synthesis and Biological Evaluation of 2-amino-3-(3', 4', 5'-trimethoxyphenylsulfonyl)-5-aryl thiophenes as a New Class of Antitubulin Agents

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Abstract: Bioisosterism represents one approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. Bioisosteres are substituents or groups that have chemical or physical similarities and that produce broadly similar biological effects. The sulfone moiety is recognized as a nonclassical bioisostere for replacement of the carbonyl group. When sulfonyl derivatives **5a-e** were compared with carbonyl compounds **4a-e**, the sulfone substitution dramatically decreased the antiproliferative activity of the series.

Key Words: Microtubule, combretastatin A-4, bioisoster, 2-aminothiophene.

1. INTRODUCTION

There is considerable interest in the discovery and development of novel small molecules able to destabilize microtubules by the inhibition of assembly of tubulin α,β dimers into polymer [1]. The microtubule system of eukaryotic cells plays important roles in regulating cell architecture, and it has an essential role in cell division, since microtubules are the key component of the mitotic spindle. Microtubules are a dynamic cellular compartment in both neoplastic and normal cells.

The discovery of natural and synthetic substances capable of interfering with the polymerization or depolymerization of microtubules has attracted much attention, since microtubules are an attractive pharmacological target for anticancer drug discovery [2]. More recently, it was established that some tubulin binding agents also target the vascular system of tumors, inducing morphological changes in the endothelial cells of the tumor blood vessels so as to occlude flow [3].

Combretastatin A-4 (CA-4, **1a**, Fig. **1**), isolated from the bark of the South African tree *Combretum caffrum* [4], is one of the well-known natural tubulin binding molecules affecting microtubule dynamics. CA-4 strongly inhibits the polymerization of tubulin by binding to the colchicine site [5]. CA-4 inhibits cell growth even at very low (nanomolar) concentrations, exhibiting inhibitory effects even on multidrug resistant (MDR) cancer cell lines [4]. The disodium phosphate prodrug of CA-4 (CA-4P, **1b**) is water-soluble, and there have been promising results as a tumor vascular targeting agent in phase II clinical trials [6]. Its structural simplicity, along with its ability to selectively damage tumor neovasculature, makes CA-4 of great interest from the medicinal chemistry point of view. For these reasons, numerous CA-4 analogues have been synthesized and evaluated for their structure-activity relationships (SAR) [7]. Replacement of the olefinic bridge of CA-4 with a carbonyl group furnished a benzophenone-type CA-4 analogue named phenstatin (**2**). This compound demonstrated interesting efficacy in a variety of tumor models, while retaining the characteristics of CA-4 [8]. The 2-aminobenzophenone derivative **3** also strongly inhibited cancer cell growth and tubulin polymerization and caused mitotic arrest, as did **2** [9].

The classical bioisosteric equivalence between benzene and thiophene prompted us recently to synthesize a series of thiophene derivatives with general formula **4**, in which a 2-aminothiophene system replaced the 2-aminobenzene moiety in the 2-amino phenstatin analogue **3**. Many of the 2-amino-3-(3',4',5'-trimethoxybenzoyl)-5-phenyl thiophene molecules with general structure **4** are potent inhibitors of tubulin polymerization and show strong antiproliferative activities against two leukemic cell lines, L1210 and K562, with accumulation of cells in the G2/M phase of the cell cycle. The most active compounds had activities comparable with those of CA-4 [10].

Bioisosterism is an important lead modification approach that has been shown to be useful to attenuate toxicity or to modify the activity of a lead, and this approach may also have a significant role in the alteration of the pharmacokinetics of a lead [11]. The objective of a bioisosteric replacement is thus to create a new compound with similar biological

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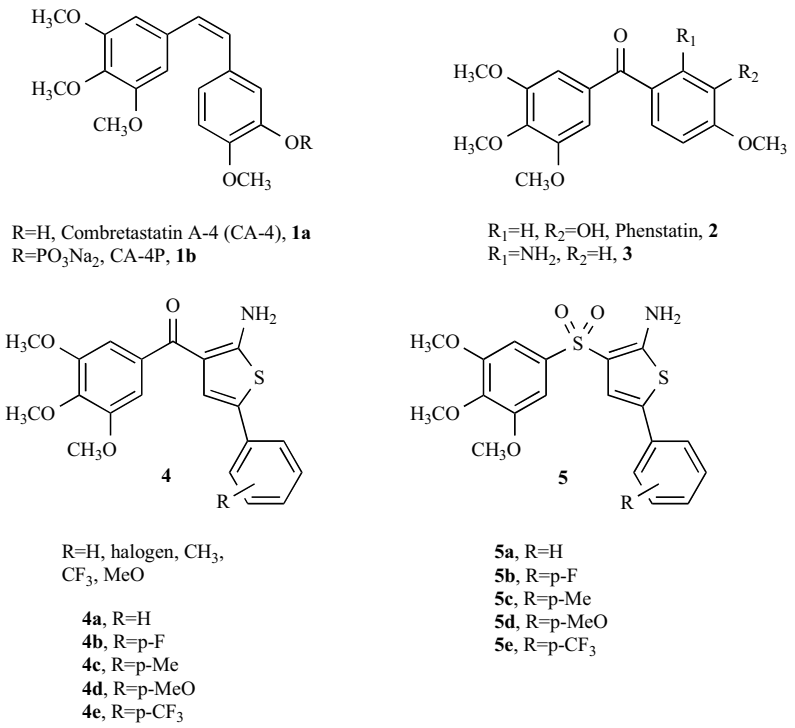


Fig. (1). Chemical structure of compounds with general structure **1-5**.

properties to the parent compound. Bioisoteres are classified as either classical or nonclassical [12].

Classical bioisosteres are those groups which have similar steric and electronic features but may or may not have a different number of atoms as compared to the structural moiety for which they are used as a replacement. In contrast, nonclassical bioisosteres do not have the same number of atoms and do not fit the steric and electronic rules of the classical isosteres, but nevertheless they do produce similar biological effects. These isosteres are believed to cause similar biological effects by imitating the spatial arrangement, electronic properties, or some other critical physiochemical property of the lead compound when bound to the biological target.

Among the most recent of numerous examples used in the bioisosterism strategy for designing new pharmacotherapeutically attractive substances nonclassic bioisosterism predominates [13,14]. Successful examples are distributed in various distinct therapeutic categories and include selective receptor antagonist or agonist drugs, enzymatic inhibitors and antimetabolites.

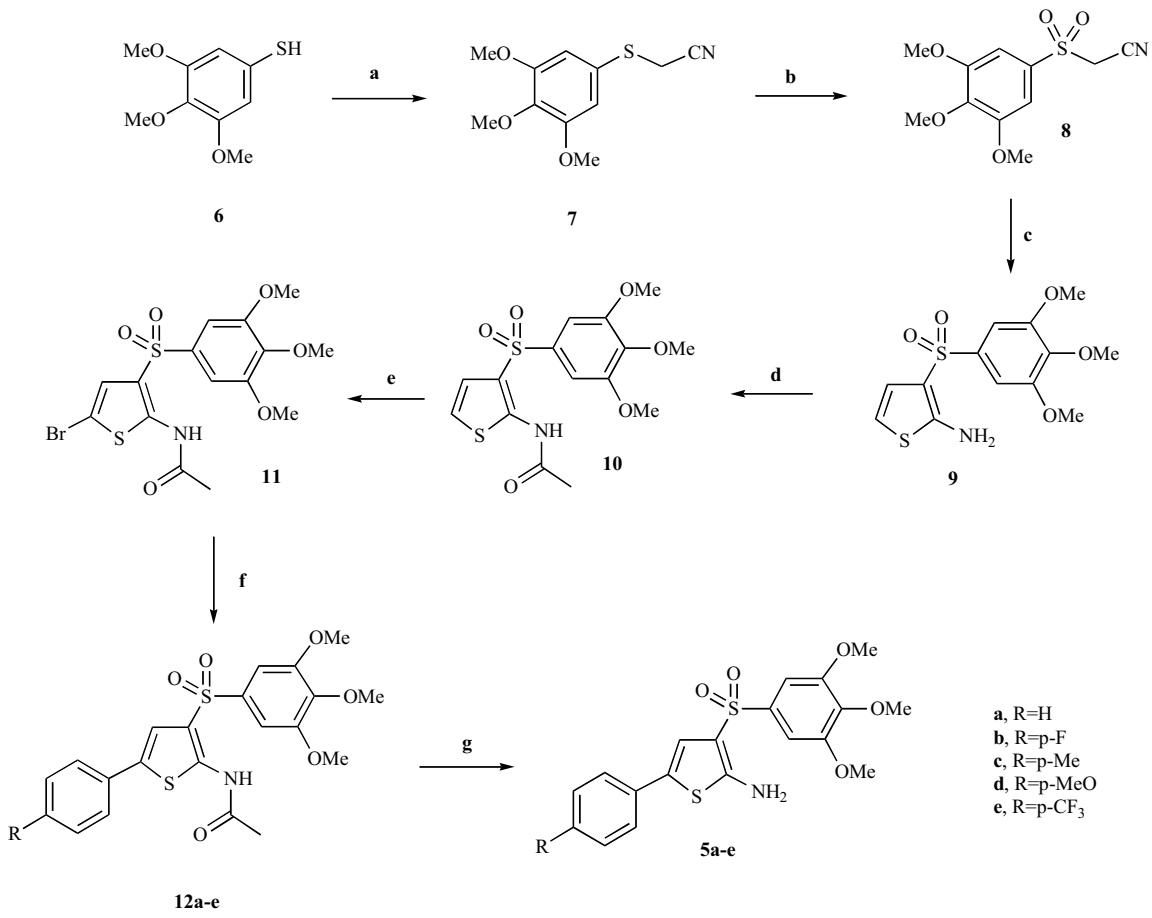
The sulfone moiety has increasingly been used as a nonclassical divalent bioisostere of the carbonyl group [12]. Building on our efforts to expand information about the SAR for the 2-amino-3-(3', 4', 5'-trimethoxybenzoyl)-5-aryl derivatives **4a-e**, we synthesized a series of 2-amino-3-(3', 4', 5'-trimethoxyphenylsulfonyl)-5-aryl bioisosteres **5a-e**, in which the 3-carbonyl moiety of compounds **4a-e** was replaced with a sulfone. These compounds were evaluated for their antiproliferative activity and for inhibitory effects on tubulin polymerization.

2. CHEMISTRY

2-Amino-3-(3',4',5'-trimethoxyphenylsulfonyl)thiophene derivatives **5a-e** were synthesized as shown in Scheme **1**. The 3,4,5-trimethoxy-benzenethiol **6** [15] was transformed into the corresponding arylthioacetonitrile **7** by treatment with chloroacetonitrile and sodium methoxide in refluxing methanol. Oxidation of sulfur derivative **7** with 3-chloroperoxybenzoic acid (MCPBA) in chloroform furnished the corresponding sulfone **8**. 2-Amino-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophene **9** was obtained by a one-step procedure (Gewald reaction [16]) applied to **8** and the dimer of α-mercaptoacetalehyde (1,4-dithiane-2,5-diol) and triethylamine (TEA) as base in ethanol. The 2-amino group was protected by acetylation, with a refluxing mixture of acetic anhydride (Ac_2O) and pyridine, yielding **10**. The regioselective bromination of **10** with *N*-bromosuccinimide (NBS) in carbon tetrachloride (CCl_4) yielded the intermediate **11**. Compounds **12a-e** were synthesized from **11** by a standard Suzuki cross-coupling reaction with appropriate aryl boronic acids under heterogeneous conditions [$Pd(PPh_3)_4$, K_2CO_3] in refluxing toluene [17]. Removal of the *N*-protected acetyl group was accomplished by the use of NaOH in a refluxing mixture of ethanol/water, to afford the final compounds **5a-e**.

3. RESULTS AND DISCUSSION

Table **1** summarizes the antiproliferative effects of thiophene derivatives **5a-e** against a panel of tumor cell lines using CA-4 (**1a**) as the reference compound. All newly synthesized derivatives **5a-e** only demonstrated growth inhibitory effects at 10-60 μM (IC_{50} values) against murine leukemia (L1210), murine mammary carcinoma (FM3A) and human T-lymphoblastoid (Molt/4 and CEM) cells, with the



Reagents.**a**: $ClCH_2CN$, MeONa, MeOH, reflux; **b**: MCPBA, DCM, rt; **c**: 1,4-dithiane-2,5-diol, TEA, EtOH, 70 °C**; d**: Ac_2O, pridine, reflux; **e**: NBS, $(C_6H_5COO)_2$, CCl_4, rx; **f**: $ArB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, PhMe, reflux, 18 h; **g**: NaOH, EtOH, reflux.

Scheme 1.

CEM cells more sensitive than the other three lines. In contrast, the IC_{50} values obtained previously with compounds **4a-e** were in the 5-90 nM range. Thus, replacement of the carbonyl function by the non classical bioisostere sulfone moiety dramatically reduced the biological activity of this class of compounds. The sulfones **5a-e** were 10^3-10^4-fold less potent than the carbonyl counterparts **4a-e**.

Compounds **5a-e** were evaluated for their *in vitro* inhibition of tubulin polymerization because of the strong inhibitory effects previously observed with **4a-e**. For comparison, CA-4 was examined in contemporaneous experiments. All new compounds were inactive as inhibitors of tubulin assembly (IC_{50}>40 μM for **5a-e** *vs*. IC_{50}=1.4 μM for CA-4).

The lack of activity of compounds **5a-e** suggests that the equivalent portion of compounds **4a-e** is critically involved in tubulin binding. This example illustrates how bioisosteric analogues can be used to identify areas of active molecules critical for formation of the pharmacophore that interacts with the biological target. The greater size of the sulfone moiety relative to the carbonyl group could be one of the factors that dramatically decreased the biological activity of **5a-e** relative to **4a-e**.

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 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.

Table 1. Inhibitory Effects of Compounds 4a-e, 5a-e and CA-4 (1a) on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A) and Human T-lymphocyte (Molt/4 and CEM) Cells

Compound	IC_{50} (μM)[a]			
	L1210	FM3A	Molt4/C8	CEM/0
4a	0.013±0.002	0.014±0.001	0.048±0.005	0.0049±0.0003
4b	0.012±0.002	0.018±0.004	0.092±0.011	0.094±0.007
4c	0.011±0.001	0.0037±0.0005	0.0088±0.0012	0.0096±0.0011
4d	0.012±0.004	0.023±0.003	0.0078±0.0006	0.0056±0.0007
4e	0.020±0.003	0.016±0.004	0.012±0.003	0.015±0.004
5a	39±3	39±4	11±1	11±1
5b	38±0	26±6	10±0	9.5±0.4
5c	41±2	64±8	15±3	10±0
5d	36±0	45±11	18±7	10±0
5e	29±4	46±3	28±7	14±1
CA-4 (1a)	0.0032±0.0007	0.042±0.003	0.0021±0.0008	0.0018±0.0004

[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.

4.1.1. (3,4,5-Trimethoxyphenylsulfanyl)-acetonitrile (7)

A solution of 3,4,5-trimethoxybenzenethiol **6** (4 g, 20 mmol) in 20 mL of methanol was treated with sodium methoxide (1.08 g, 20 mmol), stirred for 15 min at room temperature and treated slowly dropwise with chloroacetonitrile (1.5 g, 20 mmol). The mixture was stirred for 2 h at room temperature and for 6 h at reflux, filtered while warm and the filtrate evaporated. The residue was purified by silica gel column chromatography (ethyl acetate-petroleum ether, 4-6), and compound **7** was obtained as a yellow solid. Yield: 74%, mp: 81-82 °C. ^{1}H ($CDCl_3$) δ 3.53 (s, 2H); 3.84 (m, 9H); 6.83 (s, 2H).

4.1.2. (3,4,5-Trimethoxybenzenesulfonyl)-acetonitrile (8)

m-Chloroperoxybenzoic acid (MCPBA, 85% purity, 2.2 g, 20 mmol) was added portionwise to an ice-cooled solution of **7** (2.39 g, 10 mmol) in chloroform (30 mL). The reaction mixture was stirred at room temperature for 2 h, washed with saturated sodium hydrogen carbonate (3 x 10 mL), water (10 mL), brine (5 mL), and dried with Na_2SO_4, and the organic solvent was evaporated. The crude product was crystallized with 2-propanol to yield **8** as a white solid. Yield: 68%, mp: 154-155 °C. ^{1}H ($CDCl_3$) δ 3.87 (m, 9H), 4.04 (s, 2H), 7.21 (s, 2H).

4.1.3. 3-(3,4,5-Trimethoxybenzenesulfonyl)-thiophen-2-ylamine (9)

To an ice-chilled suspension of **8** (2.71 g, 10 mmol) and 1,4-dithiane-2,5-diol (thioacetaldehyde dimer) (0.76 g, 5 mmol) in EtOH (20 mL) was added TEA (0.90 mL, 10 mmol). After stirring for 6 h at rt, the solvent was evaporated and the residue dissolved with DCM (15 mL). After washing with water (2x5 mL) and brine (5 mL), the organic layer was dried and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate-dichloromethane, 1-9) to yield **9** as a brown solid. Yield: 70%, mp: 189-191 °C. ^{1}H ($CDCl_3$) δ 3.88 (m, 9H), 5.58 (bs, 2H), 6.30 (d, *J* =6.0 Hz, 1H), 6.81 (d, *J* =6.0 Hz, 1H), 7.14 (s, 2H).

4.1.4. N-[3-(3,4,5-Trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (10)

Pyridine (10 drops) was added to a stirred solution of **9** (3.3 g., 10 mmol) in Ac_2O (12 mL) at room temperature. The solution was refluxed for 2 h, poured into water, and extracted with EtOAc (50 mL). The extract was washed successively with saturated aqueous $NaHCO_3$, water, and brine and dried over Na_2SO_4. The organic phase was concentrated under vacuo. Recrystallization from 2-propanol gave **10** as a white solid. Yield: 83%, mp: 147-149 °C. ^{1}H ($CDCl_3$) δ: 2.33 (s, 3H), 3.89 (s, 9H), 6.82 (d, *J* =6.0 Hz, 1H), 6.96 (d, *J* =6.0 Hz, 1H), 7.11 (s, 2H), 10.4 (bs, 1H).

4.1.5. N-[5-Bromo-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (11)

To a suspension of compound **10** (3.71 g, 10 mmol) in CCl_4 (50 mL) was added benzoyl peroxide (120 mg, 0.5 mmol.), and the mixture was heated under reflux. A mixture of NBS (1.78 g, 10 mmol) and benzoyl peroxide (120 mg, 0.5 mmol) was added to the refluxing reaction mixture, and reflux continued for another hour. At this time, if the reaction was incomplete, additional NBS (178 mg, 1 mmol) and benzoyl peroxide (120 mg, 0.5 mmol) were added, and the reaction was refluxed for another hour. The yellow solution was then cooled to room temperature and filtered to remove precipitate, and the residue was washed with CCl_4 (5 mL). The filtrate was washed with 5% $NaHCO_3$ (15 mL), water (10 mL) and brine, and dried (Na_2SO_4). The organic phase

was concentrated. Recrystallization from 2-propanol gave **11** as a gray solid. Yield: 76%, mp: 153-155 °C. ^{1}H ($CDCl_3$) δ: 2.33 (s, 3H), 3.89 (s, 9H), 6.88 (s, 1H), 7.08 (s, 2H), 10.4 (bs, 1H).

4.1.6. General Procedure A (Suzuki coupling) for the Synthesis of Compounds (12a-e)

A mixture of thiophene derivative **11** (226 mg, 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.

4.1.7. N-[5-Phenyl-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (12a)

Following general procedure A, compound **12a** was purified by column chromatography eluting with petroleum ether-ethyl acetate (1:1). Yellow oil, yield: 95%. ^{1}H ($CDCl_3$) δ: 2.35 (s, 3H), 3.89 (m, 9H), 7.09 (s, 1H), 7,13 (s, 2H), 7.32 (m, 5H), 10.4 (bs, 1H).

4.1.8. N-[5-(4-Fluorophenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (12b)

Following general procedure A, compound **12b** was purified by column chromatography eluting with petroleum ether-ethyl acetate (6:4). Yellow oil, yield: 86%. ^{1}H NMR ($CDCl_3$) δ: 2.35 (s, 3H), 3.89 (m, 9H), 7.00 (s, 1H), 7.05 (m, 2H), 7.12 (s, 2H), 7.45 (m, 2H), 10.4 (bs, 1H).

4.1.9. N-[5-p-Tolyl-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (12c)

Following general procedure A, compound **12c** was purified by column chromatography eluting with petroleum ether-ethyl acetate (6:4). Pale yellow solid, yield: 95%, mp: 143-145 °C. ^{1}H NMR ($CDCl_3$) δ: 2.32 (s, 3H), 2.34 (s, 3H), 3.89 (m, 9H), 7.04 (s, 1H), 7.12 (s, 2H), 7.14 (m, 4H), 10.3 (bs, 1H).

4.1.10. N-[5-(4-Methoxyphenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (12d)

Following general procedure A, compound **12d** was purified by column chromatography eluting with petroleum ether-ethyl acetate (6:4). Yellow cream oil, yield: 90%. ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H), 3.81 (s, 3H), 3.88 (m, 9H), 6.86 (m, 4H), 6.97 (s, 1H), 7.12 (s, 2H), 10.3 (bs, 1H).

4.1.11. N-[5-(4-Trifluoromethylphenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-yl]-acetamide (12e)

Following general procedure A, compound **12e** was purified by column chromatography eluting with dichloromethane-ethyl acetate (8:2). White solid, yield: 96%, mp: 193-195 °C. ^{1}H NMR ($CDCl_3$) δ: 2.36 (s, 3H), 3.89 (m, 9H), 7.12 (s, 2H), 7.17 (s, 1H), 7.60 (s, 4H), 10.4 (bs, 1H).

4.1.12. General Procedure B for the Synthesis of Compounds (5a-e)

A mixture of one of compounds **12a-e** (0.5 mmol), 1 N aqueous NaOH (0.5 mL, 0.5 mmol) and EtOH (10 mL) was refluxed for 5 h and then concentrated in vacuo. The residue was diluted with EtOAc, and the mixture was washed successively with water and brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by flash chromatography on silica gel using the indicated solvent mixture as eluent.

4.1.13. 5-Phenyl-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-ylamine (5a)

Following general procedure B, compound **5a** was purified by column chromatography eluting with petroleum ether-ethyl acetate (1:1). Brown solid, yield: 64%, mp: 60-62 °C. ^{1}H NMR ($CDCl_3$) δ: 3.88 (s, 3H), 3.91 (s, 6H), 7.01 (s, 1H), 7.17 (s, 2H), 7.29 (m, 5H). Anal. $C_{19}H_{19}NO_5S_2$ (C, H, N).

4.1.14. 5-(4-Fluorophenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-ylamine (5b)

Following general procedure B, compound **5b** was purified by column chromatography eluting with dichloromethane-ethyl acetate (9.8:0.2). Brown solid, yield: 88%, mp: 175-177 °C. ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 3.90 (s, 6H), 6.92 (s, 1H), 6.98 (m, 2H), 7.16 (s, 2H), 7.29 (m, 2H). Anal. $C_{19}H_{18}FNO_5S_2$ (C, H, N).

4.1.15. 5-p-Tolyl-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-ylamine (5c)

Following general procedure B, compound **5c** was purified by column chromatography eluting with methylene chloride-ethyl acetate (9.8:0.2). Brown solid, yield: 73%, mp: 75-77 °C. ^{1}H NMR ($CDCl_3$) δ: 2.32 (s, 3H), 3.87 (m, 9H), 6.95 (s, 1H), 7.10 (m, 4H), 7.17 (s, 2H). Anal. $C_{20}H_{21}NO_5S_2$ (C, H, N).

4.1.16. 5-(4-Methoxyphenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-ylamine (5d)

Following general procedure B, compound **5d** was purified by column chromatography eluting with petroleum ether-ethyl acetate (9:1). Brown pale solid, yield: 53%, mp: 132-134 °C. ^{1}H NMR ($CDCl_3$) δ: 3.80 (s, 3H), 3.87 (m, 9H), 6.83 (m, 4H), 6.87 (s, 1H), 7.17 (s, 2H). Anal. $C_{20}H_{21}NO_6S_2$ (C, H, N).

4.1.17. 5-(4-Trifluoromethylphenyl)-3-(3,4,5-trimethoxybenzenesulfonyl)-thiophen-2-ylamin (5e)

Following general procedure A, compound **5e** was purified by column chromatography eluting with methylene chloride-ethyl acetate (9.8:0.2). Gray solid, yield: 78%, mp: 143-145 °C. ^{1}H NMR ($CDCl_3$) δ: 3.87 (s, 3H), 3.89 (m, 6H), 7.11 (s, 1H), 7.17 (s, 2H), 7.42 (m, 4H). Anal. $C_{20}H_{18}F_3NO_5S_2$ (C, H, N).

5. BIOLOGICAL EVALUATION

5.1. Inhibition of Growth of Tumor Cells

Murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphocyte Molt 4 and CEM 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

Bovine brain tubulin was purified as described previously [18]. To evaluate the effect of the compounds on tubulin assembly *in vitro* [19], 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 after 20 min incubation. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50%.

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