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ORGANOMETALLICS
Article

Synthesis and Characterization of New Organometallic Benzo[*b*]thiophene Derivatives with Potential Antitumor Properties

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The incorporation of organometallic moieties into the structure of known active drugs to improve their therapeutic properties has gained considerable interest in recent years. The benzo[*b*]thiophene derivative raloxifene is a selective estrogen receptor modulator (SERM) that has been found to decrease breast cancer risk in postmenopausal women compared to placebo. The current data suggest that, in the postmenopausal setting, raloxifene may have the benefits of the widely used tamoxifen with fewer side effects. As part of a program designed toward the synthesis and biological screening of organometallic benzo[*b*]thiophene derivatives inspired by the structure of raloxifene, we have prepared a series of 2-benzoyl-3-ferrocenylbenzo[*b*]thiophenes where the benzoyl substituent contains terminal tertiary alkylamino groups, expected to ensure affinity to the estrogen receptor. The synthetic strategy and full characterization (NMR, MS, X-ray diffraction, cyclic voltammetry) of the new ferrocenylbenzo[*b*]thiophenes is reported herein. Moreover, the new 2-benzoyl-3-ferrocenylbenzo[*b*]thiophene derivatives were tested for their cytotoxic properties against several human tumor cell lines. All the test compounds showed considerable cytotoxic activity; among these, [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperazin-1-yl)methylphenyl]methanone (compound **13**) is of note, showing IC_{50} values in the low-micromolar range and more than 1 order of magnitude lower than those of the reference compound, cisplatin. In addition, chemosensitivity tests on resistant phenotypes indicated that compound **13** elicited no cross-resistance with cisplatin, besides not being a potential multidrug-resistant (MDR) substrate. Moreover, caspase-3 activation analyses revealed that **13** induced a caspase-3-dependent apoptotic cell-death mechanism. Taken together, these data suggest that the new 2-benzoyl-3-ferrocenylbenzo[*b*]thiophenes, in particular compound **13**, have potentially useful antitumor properties.

Introduction

In the last two decades, bioorganometallic chemistry has become a rapidly growing field, which has expanded the classical scope of organometallic chemistry into biology and medical applications.[1] In particular, the topic of organometallic pharmaceuticals is of emergent significance, and although efforts have predominantly been directed toward the evaluation of organometallics as anticancer agents, other targets for organometallic therapeutic agents include parasitic, viral, and microbial diseases.[2] One such example is the chloroquine analogue ferroquine, a new antimalarial drug candidate with a low toxicity profile and no cross-resistance with chloroquine, which reached phase IIb clinical trials in the fall of 2007.[3]

The crucial event in the launching of metal-based drugs is widely considered to be the discovery of the antitumoral effects of cisplatin.[4] Ever since its approval in 1978 for the treatment of testicular and ovarian cancer, this compound has been used extensively in cancer therapy. However,

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cisplatin has numerous shortcomings, including induced cellular resistance, severe toxicity, and a spectrum of activity limited to a narrow range of tumor types.[5] This has prompted considerable efforts to develop novel metal-based anticancer agents that could circumvent the limitations of cisplatin. As a result, thousands of platinum-based compounds have been synthetized and screened for toxicity and anticancer activity, although very few have been approved for clinical use.[5,6] The search for other metal-based anticancer compounds that could circumvent the drawbacks associated with platinum drugs has been pursued concurrently; representative examples include titanocene and ferrocene derivatives,[2] as well as ruthenium complexes.[2,7] Ferrocene derivatives, in particular, due to their stability in aerobic aqueous media, synthetic versatility, and favorable electrochemistry, are among the most widely considered species for biological applications.[8]

A pioneering strategy regarding the medicinal use of organometallic species involves the incorporation of organometallic moieties into the structures of known active drugs to improve their therapeutic properties.[9] One of the most intriguing examples of this approach is the chemical modification of the estrogen antagonist tamoxifen (**1**, Scheme 1) to contain a series of metallocene fragments, which resulted in varied, and in some instances unforeseen, biological properties.[10] In particular, the inclusion of a ferrocenyl fragment into the structure of the active tamoxifen metabolite, 4-hydroxytamoxifen (**2**, Scheme 1), imparted cytotoxic characteristics to the final product, hydroxyferrocifen (**3**, Scheme 1), without hampering the intrinsic antiestrogenicity of the tamoxifen vector.[11] In fact, hydroxyferrocifen displayed antiproliferative effects *in vitro* on both estrogen receptor (ER)-positive and ER-negative breast cancer cell lines, which indicated an ability to act through both hormone-dependent and hormone-independent mechanisms.[11]

Tamoxifen is currently an important adjuvant chemotherapeutic agent for the treatment of all stages of hormone-dependent breast cancer[12] and also a chemoprotective agent for the prevention of the disease in high-risk women.[13] Despite being beneficial for breast cancer treatment and prevention, tamoxifen is known to increase the risk of endometrial cancer and thromboembolic events in women.[14] These limitations have prompted the search for additional selective estrogen receptor modulators (SERMS). An ideal SERM should have improved effects on breast cancer, osteoporosis, and coronary heart disease, but fewer toxic side effects, such as endometrial cancer induction.

The 2-arylbenzothiophene raloxifene (**4**, Scheme 1) is a SERM originally developed, and approved, for the prevention and treatment of postmenopausal osteoporosis. While raloxifene is an estrogen agonist in bone tissue, resulting in potent inhibition of the loss of volumetric bone mineral density, it acts as an antiestrogen in mammary tissue and has minimal effects on the endometrium.[15] Raloxifene administration has been found to decrease breast cancer risk in postmenopausal women compared to placebo, and the current data suggest that, in the postmenopausal setting, it may have the benefits of tamoxifen with fewer side effects.[16] Based upon these observations, and with the aim of exploring the concept of target-oriented organometallic drugs, we have recently undertaken a program designed toward the synthesis and biological screening of organometallic benzo[*b*]thiophene derivatives inspired by the structure of raloxifene. These species have been designed to assess the possible combination of SERM properties associated with the benzo[*b*]thiophene backbone with potential cytotoxicity imparted by the organometallic fragment. We report herein the synthesis and characterization of the first representatives of this class, a series of 2-benzoyl-3-ferrocenylbenzo[*b*]thiophenes where the benzoyl substituent contains terminal tertiary alkylamino groups, expected to ensure affinity to the ER.[17] As an initial step in ascertaining the antitumor potential of the new compounds, their cytotoxic properties were evaluated against several human tumor cell lines, with all test compounds displaying considerable cytotoxic activity. In addition, we report the results of chemosensitivity tests on

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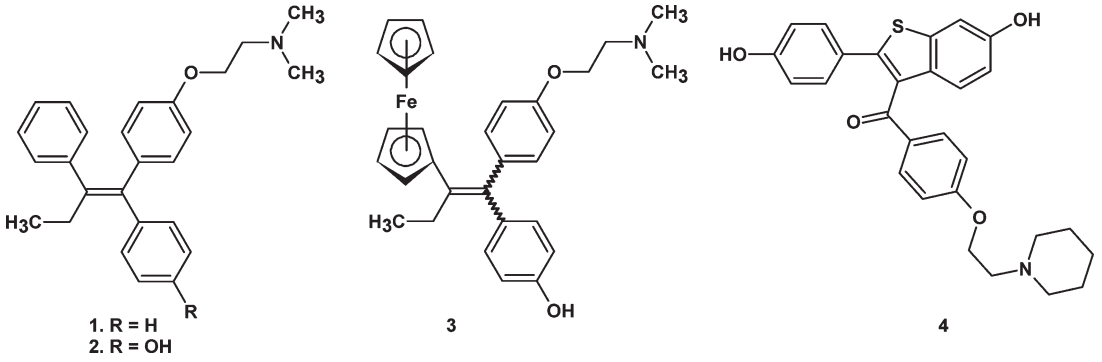
Scheme 1. Structures of Tamoxifen (1), 4-Hydroxytamoxifen (2), Ferrocifen (3), and Raloxifene (4)



resistant phenotypes conducted with all the new ferrocenylbenzo[*b*]thiophenes, as well as caspase-3 activation analyses, performed with the most active compound, [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperazin-1-yl)methylphenyl]methanone (compound **13**). The test compounds elicited no cross-resistance with cisplatin and gave no indication of being potential multidrug-resistant (MDR) substrates; moreover, compound **13** was found to induce a caspase-3-dependent apoptotic cell-death mechanism.

Experimental Section

Chemicals and General Procedures. All commercially available chemicals were acquired from Sigma-Aldrich Química, S. A. (Madrid, Spain). All synthetic procedures were conducted in air. Whenever necessary, solvents were purified by standard methods.[18]

Microwave-assisted syntheses were conducted in a CEM Discover Benchmate microwave reactor, operating at 300 W. Melting temperatures were measured with a Leica Galen III hot stage apparatus and are uncorrected. Microanalyses (C, H, N, and S) were performed at Laboratório de Análises, Instituto Superior Técnico. Infrared (IR) spectra were recorded on a Perkin-Elmer 683 FT-IR spectrometer. Group frequencies are reported in cm^{-1}; the symbols s, m, w, and br represent strong, medium, weak, and broad bands, respectively.

Mass spectra (MS) were recorded on either a GC-TOF Micromass mass spectrometer, operated in the electron ionization (EI) mode, or a Varian 500-MS LC ion trap mass spectrometer, operated in the electrospray ionization (ESI) mode. For ESI analyses, the spray voltage was set at ±5 kV and the capillary voltage was set at 10 V. Fragment percent intensities and fragment assignments are indicated in parentheses and square brackets, respectively. High-resolution mass spectra (HRMS) were recorded on a Finnigan FT/MS 2001-DT spectrometer.

^{1}H NMR spectra were recorded in acetone-d_6, on a Bruker Avance III 300 or a Bruker Avance III 400 spectrometer, operating at 300 or 400 MHz, respectively. ^{13}C NMR spectra were recorded on the same instruments, operating at 75.47 and 100.62 MHz, respectively. Chemical shifts are reported in ppm downfield from tetramethylsilane, and coupling constants (*J*) are reported in Hz; the subscripts *ortho* and *meta* refer to *ortho* and *meta* couplings, respectively. The abbreviations used to assign specific protons and carbons include Ar (aryl, for benzothiophene), Fc (ferrocene), and Ph (*para*-substituted phenyl). Resonance and structural assignments were based on the analysis of coupling patterns, including the ^{13}C−^{1}H coupling profiles obtained in heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments, performed with standard pulse programs.

Electrochemical experiments were performed on an EG&G Princeton Applied Research potentiostat/galvanostat model 273A and controlled by a personal computer using Electrochemistry PowerSuite v2.51 data acquisition software from Princeton Applied Research. The voltammetric experiments were performed at room temperature, using a three-electrode configuration with a platinum-disk working electrode (1.0 mm diameter) probed by a Luggin capillary connected to a silver-wire pseudoreference electrode; a platinum wire auxiliary electrode was employed. The solutions under study were 1 mM in acetonitrile and 0.1 M in the supporting electrolyte (tetra-*n*-butylammonium hexafluorophosphate). The redox potentials of the ferrocenylbenzo[*b*]thiophenes were measured in the presence of ferrocene as the internal standard and are reported relative to the value of the ferrocenium/ferrocene redox couple [$E_p/2$ = 0.40 V versus the saturated calomel electrode (SCE) in acetonitrile].[19] $E_p/2$ values were determined as $(E_{pa} + E_{pc})/2$. The solutions were purged with nitrogen and kept under an inert atmosphere throughout the measurements. Reagent grade acetonitrile was dried over CaH_2 and distilled under nitrogen before use.

Syntheses. 2-Chloro-1-ferrocenylethanone (5). Ferrocene (15 g, 81 mmol) and chloroacetyl chloride (6 g, 53 mmol) were dissolved in 100 mL of dry, nitrogen-purged methylene chloride, and the solution was cooled to −15 °C. Anhydrous aluminum trichloride (7.15 g, 54 mmol) was then added slowly, for a period of 4 h, while maintaining the temperature, and the mixture was subsequently allowed to reach room temperature and stirred overnight. The reaction was then quenched with ice, and the mixture extracted with methylene chloride. The organic extracts were dried with anhydrous magnesium sulfate and evaporated to dryness, yielding a brown solid. This solid was homogenized with Celite and then purified by column chromatography on silica, using methylene chloride/*n*-hexane (1:2) as the eluent, to yield a red crystalline product (9.6 g, 37 mmol, 69%). Mp: 88−90 °C [mp 94−96 °C;[20] 92−93 °C[21]]. IR (KBr): 1679 (s, C=O) cm^{-1}. ^{1}H NMR δ 4.90 (2H, bs, Fc-**H2**+**H5**), 4.68 (2H, s, C**H**$_2$Cl), 4.66 (2H, bs, Fc-**H3**+**H4**), 4.31 (5H, s, Fc-**H1′** to **H5′**). ^{13}C NMR δ 195.0 (**C**O), 77.2 (Fc-**C1**), 73.5 (Fc-**C2**+**C5**), 70.7 (Fc-**C1′** to **C5′**), 70.1 (Fc-**C3**+**C4**), 47.1 (**C**H$_2$Cl).

1-Ferrocenyl-2-[3-(methoxyphenyl)thio]ethanone (6). Potassium *tert*-butoxide (1.5 equiv) was added to a solution of 3-methoxybenzenethiol (2 g, 14 mmol) in 30 mL of anhydrous diethyl ether, and the suspension was stirred at room temperature for 1 h. 2-Chloro-1-ferrocenylethanone (**5**, 5.6 g, 1.5 equiv) was added, and the mixture was stirred overnight. The solid materials were then removed by filtration over Celite. The

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thioether (**6**) precipitated upon cooling, and red crystals (3.33 g, 9.1 mmol, 64%) were collected. Mp: 124−126 °C (diethyl ether). IR (KBr): 1659 (s, C=O), 1588 (m), 1454 (m), 1247 (m), 1032 (m) cm^{-1}. ^1H NMR: δ 7.24 (1H, t, J 7.8, Ar-**H5**), 7.02 (2H, m, Ar-**H2**+**H4** or **H6**), 6.79 (1H, ddd, J_{ortho} 8.4, J_{meta} 2.3, J'_{meta} 0.9, Ar-**H4** or Ar-**H6**), 4.90 (2H, t, J 2.0, Fc-**H2**+**H5**), 4.61 (2H, t, J 2.0, Fc-**H3**+**H4**), 4.28 (2H, s, C**H**$_2$S), 4.27 (5H, s, Fc-**H1′** to **H5′**), 3.81 (3H, s, C**H**$_3$O). ^{13}C NMR: δ 197.9 (**C**O), 160.8 (Ar-**C3**), 138.5 (Ar-**C1**), 130.4 (Ar-**C**H), 121.5 (Ar-**C**H), 114.9 (Ar-**C**H), 112.4 (Ar-**C**H), 78.7 (Fc-**C1**), 73.1 (Fc-**C2**+**C5**), 70.5 (Fc-**C1′** to C5′ or Fc-**C3**+**C4**), 70.3 (Fc-**C1′** to **C5′** or Fc-**C3**+**C4**), 55.4 (**C**H$_3$O), 41.5 (**C**H$_2$S). MS (EI): m/z 366 (50%) [M^+], 213 (100%) [$FcCO^+$], 185 (58%) [Fc^+], 129 (27%) [Fc^+ − Fe], 121 (9%) [$CpFe^+$], 56 (4%) [Fe^+]. Anal. Calcd for $C_{19}H_{18}FeO_2S\cdot 0.5Et_2O$: C, 62.54; H, 5.75; S, 7.95. Found: C, 62.54; H, 5.59; S, 8.28.

3-Ferrocenyl-6-methoxybenzo[*b*]thiophene (7). 1-Ferrocenyl-2-[3-(methoxyphenyl)thio]ethanone (**6**, 3.33 g, 9.1 mmol) was dissolved in dry *p*-xylene (30 mL) and refluxed for 4 h with HCl-activated Amberlyst 15 resin (10% w/w). The reaction mixture was then filtered over Celite, and the solvent was evaporated under vacuum. The slightly oily orange residue was purified by flash chromatography on silica, by eluting with methylene chloride/*n*-hexane (1:4). The pure benzo[*b*]thiophene derivative (**7**) was obtained as a crystalline orange product (2.28 g, 6.5 mmol, 72%). In subsequent experiments, the reaction was further optimized by subjecting solutions of **6** (40−60 $mg\cdot mL^{-1}$) in dry *p*-xylene, in the presence of Amberlyst 15 resin (ca. 60 $mg\cdot mL^{-1}$), to microwave irradiation for 20 min at 300 W; under these conditions, **7** was isolated in 90−99% yield. Mp: 85−87 °C. IR (KBr): 3091 (m), 2930 (m), 1602 (s), 1471 (s), 1266 (s), 1233 (s) cm^{-1}. ^1H NMR: δ 8.31 (1H, d, J_{ortho} 9.0, Ar-**H4**), 7.53 (1H, d, J_{meta} 2.4, Ar-**H7**), 7.41 (1H, s, Ar-**H2**), 7.15 (1H, dd, J_{ortho} 9.0, J_{meta} 2.4, Ar-**H5**), 4.73 (2H, t, J 1.8, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.38 (2H, t, J 1.8, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.15 (5H, s, Fc-**H1′** to **H5′**), 3.92 (3H, s, C**H**$_3$O). ^{13}C NMR: δ 157.4 (Ar-**C6**), 142.1 (Ar-**C7a**), 133.6 (Ar-**C3**), 131.8 (Ar-**C3a**), 124.9 (Ar-**C4**), 118.7 (Ar-**C2**), 113.8 (Ar-**C5**), 105.1 (Ar-**C7**), 81.3 (Fc-**C1**), 68.9 (Fc-**C1′** to **C5′**), 68.1 (Fc-**C3**+**C4** or Fc-**C2**+**C5**), 67.1 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 54.8 (**C**H$_3$O). MS (EI): m/z 348 (100%) [M^+], 333 (29%) [$M^+ - CH_3$], 305 (17%) [$M^+ - CH_3 - CO$], 239 (5%) [$M^+ - CH_3 - CO - Cp - H$], 227 (6%) [M^+ − FeCp], 215 (6%) [$M^+ - CH_3 - CO - Cp - C_2H$], 184 (7%) [$M^+ - CH_3 - CO$ − FeCp], 152 (10%) [$M^+ - CH_2O$ − FeCp − CH=S], 139 (6%) [$M^+ - CH_3 - CO$ − FeCp − CH=S], 121 (7%) [$FeCp^+$], 56 (2.5%) [Fe^+]. Anal. Calcd for $C_{19}H_{16}FeOS\cdot 0.5MeOH$: C, 64.30; H, 4.98; S, 8.80. Found: C, 64.33; H, 5.06; S, 8.47.

[4-(Chloromethyl)phenyl][3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl]methanone (8). The benzo[*b*]thiophene **7** (2.5 g, 7.2 mmol) was dissolved in anhydrous diethyl ether and lithiated at −70 °C with a stoichiometric amount of *n*-butyllithium (2 M solution in *n*-pentane). The reaction mixture was allowed to reach room temperature, stirred overnight, and then evaporated to dryness and washed with *n*-hexane; the resulting yellow salt was used without further purification. The lithiated benzo-[*b*]thiophene was redissolved in anhydrous diethyl ether (20 mL) and added slowly to a stirred solution of 4-chloromethyl-benzoyl chloride (1.35 g, 7.1 mmol) in 50 mL of dry diethyl ether, kept at −60 °C. An immediate color change, from yellow to dark red, was observed. The reaction mixture was allowed to warm slowly to room temperature, and stirring was continued for one additional hour. The mixture was then filtered and the solution evaporated to dryness. The dark red oily residue was purified by column chromatography (silica, methylene chloride) to yield dark red crystals of compound **8** (1.8 g, 3.6 mmol, 51%). Mp: 70−72 °C. IR (KBr): 3090 (w), 2933 (w), 1626 (m), 1602 (s), 1452 (m), 1267 (m), 1229 (s) cm^{-1}. ^1H NMR: δ 8.77 (1H, d, J_{ortho} 8.8, Ar-**H4**), 7.60 (1H, J_{meta} 2.4, Ar-**H7**), 7.59 (2H, d, J_{ortho} 8.4, Ph-**H2**+**H6**), 7.36 (2H, d, J_{ortho} 8.4, Ph-**H3**+**H5**), 7.29 (1H, dd, J_{ortho} 8.8, J_{meta} 2.4, Ar-**H5**), 4.69 (2H, s, C**H**$_2$Cl), 4.39 (2H, t, J 1.8, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.16 (2H, t, J 1.8, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.11 (5H, s, Fc-**H1′** to **H5′**), 3.99 (3H, s, C**H**$_3$O). ^{13}C NMR: δ 192.3 (**C**O), 161.0 (Ar-**C6**), 144.0 (Ph-**C4** or Ar-**C7a**), 143.8 (Ar-**C7a** or Ph-**C4**), 139.3 (Ar-**C3** or Ph-**C1**), 139.1 (Ph-**C1** or Ar-**C3**), 135.6 (Ar-**C2**), 134.6 (Ar-**C3a**), 131.5 (Ph-**C2**+**C6**), 130.0 (Ph-**C3**+**C5**), 128.8 (Ar-**C4**), 116.7 (Ar-**C5**), 106.1 (Ar-**C7**), 82.0 (Fc-**C1**), 71.7 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 71.0 (Fc-**C1′** to C5′), 70.2 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 56.9 (**C**H$_3$O), 46.8 (**C**H$_2$Cl). MS (ESI): m/z 502 [$M^+(^{37}Cl)$], 500 (97%) [$M^+(^{35}Cl)$], 437 [$M^+(^{37}Cl)$ − Cp], 435 (49%) [$M^+(^{35}Cl)$ − Cp], 390 (26%) [$MH^+ - Cl - CH_3OH$ − C=S], 348 (100%) [$MH^+ - ClCH_2$-C_6H_4CO]. Anal. Calcd for $C_{27}H_{21}ClFeO_2S\cdot LiCl$: C, 59.70; H, 3.90; S, 5.90. Found: C, 59.56; H, 4.06; S, 6.30.

[3-Ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(dimethyl-aminomethyl)phenyl]methanone (9). Dimethylamine (2 M in THF, 0.8 mL, 1.6 mmol) was added to a solution of the benzo[*b*]thiophene **8** (250 mg, 0.5 mmol) in DMF (10 mL), and the mixture was stirred overnight. Following dilution with approximately 10 volumes of water and extraction with methylene chloride, the organic extracts were dried with anhydrous sodium sulfate, and the ensuing dark red oil was purified by plc (silica, methylene chloride) to yield a red crystalline solid (110 mg, 0.22 mmol, 43%). Mp: 97−98 °C. IR (KBr): 2939 (m), 1705 (m), 1624 (s, CO), 1601 (s), 1521 (m), 1453 (s), 1338 (m), 1280 (s), 1231 (s) cm^{-1}. ^1H NMR: δ 8.75 (1H, d, J_{ortho} 9.1, Ar-**H4**), 7.52 (2H, d, J_{ortho} 7.9, Ph-**H2**+**H6**), 7.59 (1H, d, J_{meta} 2.2, Ar-**H7**), 7.28 (1H, dd, J_{ortho} 9.1, J_{meta} 2.2, Ar-**H5**), 7.22 (2H, d, J_{ortho} 7.9, Ph-**H3**+**H5**), 4.36 (2H, bs, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.16 (2H, bs, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.13 (5H, s, Fc-**H1′** to **H5′**), 3.98 (3H, s, C**H**$_3$O), 3.40 (2H, s, C**H**$_2$N), 2.22 (6H, s, (C**H**$_3$)$_2$N). ^{13}C NMR: δ 191.0 (**C**O), 159.2 (Ar-**C6**), 144.2 (Ph-**C4**), 142.2 (Ar-**C7a**), 137.2 (Ar-**C3** or Ph-**C1**), 136.7 (Ar-**C3** or Ph-**C1**), 134.4 (Ar-**C2**), 133.0 (Ar-**C3a**), 129.5 (Ph-**C2**+**C6**), 128.4 (Ph-**C3**+**C5**), 127.0 (Ar-**C4**), 115.0 (Ar-**C5**), 104.4 (Ar-**C7**), 80.5 (Fc-**C1**), 70.0 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 69.0 (Fc-**C1′** to **C5′**), 68.4 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 63.2 (**C**H$_2$N), 55.2 (**C**H$_3$O), 44,5 [(**C**H$_3$)$_2$N)]. MS (ESI): m/z 510 (100%) [MH]$^+$, 465 (47%) [MH − $(CH_3)_2NH]^+$, 444 (19%) [MH − CpH]$^+$, 359 (17%) [MH − $(CH_3)_2NH - CH_3 - CO - Cp + 2H]$, 269 (17%) [$MH_2$ − $C_6H_4CH_2N(CH_3)_2 - CH_3 - CO$ − Cp]. Anal. Calcd for $C_{29}H_{27}FeNO_2S\cdot 0.5HCl$: C, 66.01; H, 5.25; N, 2.65; S, 6.08. Found: C, 66.23; H, 5.47; N, 2.44; S, 6.33.

[3-Ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperidin-1-yl)methylphenyl]methanone (10). This compound was prepared and purified as described for **9**, using benzo[*b*]thiophene **8** (250 mg, 0.5 mmol) and 200 μL (2 mmol) of piperidine. Compound **10** was isolated as a red crystalline solid (170 mg, 0.31 mmol, 62%). Mp: 97−99 °C. IR (KBr): 2933 (m), 1627 (s, CO), 1602 (s), 1521 (m), 1451 (s), 1341 (m), 1268 (s), 1233 (s) cm^{-1}. ^1H NMR: δ 8.76 (1H, d, J_{ortho} 9.1, Ar-**H4**), 7.60 (1H, d, J_{meta} 2.4, Ar-**H7**), 7.51 (2H, d, J_{ortho} 8.3, Ph-**H2**+**H6**), 7.28 (1H, dd, J_{ortho} 9.1, J_{meta} 2.4, Ar-**H5**), 7.22 (2H, d, J_{ortho} 8.3, Ph-**H3**+**H5**), 4.36 (2H, t, J 1.9, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.13 (2H, t, J 1.9, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.11 (5H, s, Fc-**H1′** to **H5′**), 3.99 (3H, s, C**H**$_3$O), 3.41 (2H, s, C**H**$_2$N), 2.31 (4H, m, piperidine-C2**H**$_2$+C6**H**$_2$), 1.55 (4H, m, piperidine-C3**H**$_2$+C5**H**$_2$), 1.44 (2H, m, piperidine-C4**H**$_2$). ^{13}C NMR: δ 191.7 (**C**O), 160.0 (Ar-**C6**), 145.0 (Ph-**C4**), 142.9 (Ar-**C7a**), 137.9 (Ar-**C3** or Ph-**C1**), 137.2 (Ar-**C3** or Ph-**C1**), 135.2 (Ar-**C2**), 133.7 (Ar-**C3a**), 130.1 (Ph-**C2**+**C6**), 129.1 (Ph-**C3**+**C5**), 127.8 (Ar-**C4**), 115.6 (Ar-**C5**), 105.1 (Ar-**C7**), 81.2 (Fc-**C1**), 70.7 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 70.0 (Fc-**C1′** to **C5′**), 69.0 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 63.5 (**C**H$_2$N), 55.7 (**C**H$_3$O), 54.8 (piperidine-**C2**+**C6**), 26.6 (piperidine-**C3**+**C5**), 24.9 (piperidine-**C4**). MS (ESI): m/z 550 (100%) [MH]$^+$, 465 (17%) [MH − piperidine]$^+$, 437 (8%) [$MH - C_5H_{10}NCH_2 - CH_3$]$^+$. Anal. Calcd for $C_{32}H_{31}FeNO_2S\cdot 0.2HCl$: C, 69.03; H, 5.65; N, 2.52; S, 5.76. Found: C, 68.72; H, 5.78; N, 2.53; S, 6.19.

[3-Ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(pyrrolidin-1-yl)methylphenyl]methanone (11). This compound was prepared and purified as described for **9**, using benzo[*b*]thiophene **8** (250 mg, 0.5 mmol) and 170 μL (2 mmol) of pyrrolidine. Compound **11** was isolated as a red crystalline solid (180 mg, 0.35 mmol, 67%). Mp: 82−84 °C. IR (KBr): 2963 (m), 2778 (m), 1636 (s, CO), 1602 (s), 1525 (m), 1456 (m), 1340 (m), 1264 (s), 1231 (s), 1107 (s), 1019 (s), 806 (s) cm^{-1}. ^1H NMR: δ 8.76 (1H, d, J_{ortho} 9.0, Ar-**H4**), 7.60 (1H, d, J_{meta} 2.4, Ar-**H7**), 7.52 (2H, d, J_{ortho} 8.4, Ph-**H2**+**H6**), 7.28 (1H, dd, J_{ortho} 9.0, J_{meta} 2.4, Ar-**H5**), 7.24 (2H, d, J_{ortho} 8.4, Ph-**H3**+**H5**), 4.37 (2H, t, *J* 1.8, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.15 (2H, t, *J* 1.8, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.11 (5H, s, Fc-**H1′** to **H5′**), 3.99 (3H, s, C$\mathbf{H_3}$O), 3.58 (2H, s, C$\mathbf{H_2}$N), 2.41 (4H, m, pyrrolidine-C2$\mathbf{H_2}$+C5$\mathbf{H_2}$), 1.76 (4H, m, pyrrolidine-C3$\mathbf{H_2}$+C4$\mathbf{H_2}$). ^{13}C NMR: δ 191.4 (**C**O), 159.6 (Ar**C6**), 145.3 (Ph-**C4**), 142.6 (Ar-**C7a**), 137.5 (Ar-**C3** or Ph-**C1**), 136.9 (Ar-**C3** or Ph-**C1**), 134.7 (Ar-**C2**), 133.4 (Ar-**C3a**), 129.9 (Ph-**C2**+**C6**), 128.5 (Ph-**C3**+**C5**), 127.5 (Ar-**C4**), 115.4 (Ar-**C5**), 104.9 (Ar-**C7**), 80.9 (Fc-**C1**), 70.4 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 69.7 (Fc-**C1′** to **C5′**), 68.8 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 60.0 (**C**H_2N), 55.6 (**C**H_3O), 54.0 (pyrrolidine-**C2**+**C5**), 23.7 (pyrrolidine-**C3**+**C4**). MS (ESI): *m*/*z* 536 (100%) $[MH]^+$, 465 (21%) $[MH - \text{pyrrolidine}]^+$, 437 (8.5%) $[MH - C_4H_8NCH_2 - CH_3]^+$, 262 (9%) $[MH_2 - C_4H_8NCH_2C_6H_4CO - C{=}S - CH_3 - CO]^+$. HRMS Calcd for $C_{31}H_{29}{}^{56}FeNO_2{}^{32}S$: 535.127387. Found: 535.12827.

[3-Ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(morpholin-4-yl)methylphenyl]methanone (12). This compound was prepared and purified as described for **9**, using benzo[*b*]thiophene **8** (250 mg, 0.5 mmol) and 350 μL (4 mmol) of morpholine. Compound **12** was isolated as a red crystalline solid (200 mg, 0.36 mmol, 73%). Mp: 141−142 °C. IR (KBr): 2961 (m), 2941 (m), 2860 (m), 1622 (s, CO), 1601 (s), 1453 (s), 1340 (m), 1277 (s), 1265 (s), 1231 (s), 1105 (s), 1008 (s), 830 (s) cm^{-1}. ^1H NMR: δ 8.76 (1H, d, J_{ortho} 9.0, Ar-**H4**), 7.60 (1H, d, J_{meta} 2.4, Ar-**H7**), 7.51 (2H, d, J_{ortho} 8.1, Ph-**H2**+**H6**), 7.28 (1H, dd, J_{ortho} 9.0, J_{meta} 2.4, Ar-**H5**), 7.23 (2H, d, J_{ortho} 8.1, Ph-**H3**+**H5**), 4.36 (2H, t, *J* 1.8, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.12 (2H, t, *J* 1.8, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.10 (5H, s, Fc-**H1′** to **H5′**), 3.99 (3H, s, C$\mathbf{H_3}$O), 3.62 (4H, t, *J* 4.5, morpholine-C2$\mathbf{H_2}$+C6$\mathbf{H_2}$), 3.46 (2H, s, C$\mathbf{H_2}$N), 2.35 (4H, t, *J* 4.5, morpholine-C3$\mathbf{H_2}$+C5$\mathbf{H_2}$). ^{13}C NMR: δ 191.0 (**C**O), 159.2 (Ar**C6**), 143.2 (Ph-**C4**), 142.2 (Ar-**C7a**), 137.3 (Ar-**C3** or Ph-**C1**), 136.7 (Ar-**C3** or Ph-**C1**), 134.4 (Ar-**C2**), 133.0 (Ar-**C3a**), 129.4 (Ph-**C2**+**C6**), 128.5 (Ph-**C3**+**C5**), 127.0 (Ar-**C4**), 114.9 (Ar-**C5**), 104.4 (Ar-**C7**), 80.5 (Fc-**C1**), 70.0 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 69.3 (Fc-**C1′** to **C5′**), 68.3 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 66.5 (morpholine-**C2**+**C6**), 62.4 (**C**H_2N), 55.2 (**C**H_3O), 53.4 (morpholine-**C3**+**C5**). MS (ESI): *m*/*z* 552 (100%) $[MH]^+$, 486 (10%) $[MH - CpH]^+$, 465 (23%) $[MH - \text{morpholine}]^+$, 437 (7%) $[MH - OC_4H_8NCH_2 - CH_3]^+$, 334 (13%) $[MH_2 - CH_3OH - FeCp_2]^+$, 278 (70%) $[MH - OC_4H_8NCH_2C_6H_4 - CH_3OH - CpH]^+$. Anal. Calcd for $C_{31}H_{29}FeNO_3S \cdot 0.2HCl$: C, 66.63; H, 5.27; N, 2.51; S, 5.74. Found: C, 66.99; H, 5.30; N, 2.50; S, 5.93.

[3-Ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperazin-1-yl)methylphenyl]methanone (13). This compound was prepared and purified as described for **9**, using benzo[*b*]thiophene **8** (250 mg, 0.5 mmol) and 170 mg (2 mmol) of piperazine. Compound **13** was isolated as a red crystalline solid (150 mg, 0.27 mmol, 54%). Mp: 138−140 °C. IR (KBr): 3434 (br, NH), 2933 (m), 2813 (m), 1619 (s, CO), 1602 (s), 1453 (m), 1384 (s), 1340 (m), 1276 (m), 1230 (s), 1106 (m), 824 (m) cm^{-1}. ^1H NMR: δ 8.75 (1H, d, J_{ortho} 9.1, Ar-**H4**), 7.59 (1H, d, J_{meta} 2.4, Ar-**H7**), 7.50 (2H, d, J_{ortho} 8.4, Ph-**H2**+**H6**), 7.28 (1H, dd, J_{ortho} 9.1, J_{meta} 2.4, Ar-**H5**), 7.22 (2H, d, J_{ortho} 8.4, Ph-**H3**+**H5**), 4.35 (2H, t, *J* 1.9, Fc-**H2**+**H5** or Fc-**H3**+**H4**), 4.12 (2H, t, *J* 1.9, Fc-**H3**+**H4** or Fc-**H2**+**H5**), 4.10 (5H, s, Fc-**H1′** to **H5′**), 3.98 (3H, s, C$\mathbf{H_3}$O), 3.41 (2H, s, C$\mathbf{H_2}$N), 2.91 (4H, bs, piperazine-C3$\mathbf{H_2}$+C5$\mathbf{H_2}$), 2.80 (4H, bs, piperazine-C2$\mathbf{H_2}$+C6$\mathbf{H_2}$). ^{13}C NMR: δ 191.0 (**C**O), 159.2 (Ar**C6**), 143.8 (Ph-**C4**), 142.3 (Ar-**C7a**), 137.3 (Ar-**C3** or Ph-**C1**), 136.6 (Ph-**C1** or Ar-**C3**), 134.5 (Ar-**C2**), 133.0 (Ar-**C3a**), 129.5 (Ph-**C2**+**C6**), 128.5 (Ph-**C3**+**C5**), 127.1 (Ar-**C4**), 115.0 (Ar-**C5**), 104.4 (Ar-**C7**), 80.5 (Fc-**C1**), 70.0 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 69.3 (Fc-**C1′** to **C5′**), 68.4 (Fc-**C2**+**C5** or Fc-**C3**+**C4**), 62.8 (**C**H_2N), 55.2 (**C**H_3O), 54.2 (piperazine-**C2**+**C6**), 45.9 (piperazine-**C3**+**C5**). MS (ESI): *m*/*z* 551 (100%) $[MH]^+$, 485 (26%) $[MH - CpH]^+$, 465 (13%) $[MH - \text{piperazine}]^+$, 437 (6%) $[MH - NHC_4H_8NCH_2 - CH_3]^+$. HRMS Calcd for $C_{31}H_{30}{}^{56}FeN_2O_2{}^{32}S$: 550.138266. Found: 550.137497.

X-ray Crystallographic Analyses. X-ray crystallographic data for compounds **6**, **11**, and **12** were collected using an area detector diffractometer (Bruker AXS-KAPPA APEX II) equipped with an Oxford Cryosystem open-flow nitrogen cryostat at 150 K and graphite-monochromated Mo Kα (λ = 0.710 73 Å) radiation. Cell parameters were retrieved using Bruker SMART software and refined using Bruker SAINT[22] on all observed reflections. Absorption corrections were applied using SADABS.[23] The structures were solved by direct methods using SIR 97[24] and refined using full-matrix least-squares refinement against F^2 using SHELXL-97.[25] All the programs are included in the WINGX package (version 1.64.05).[26] All non-hydrogen atoms were refined anisotropically; the hydrogen atoms were inserted in idealized positions, riding on the parent C atom, except for compound **6**, where they were localized in the electron density map. Drawings were made with ORTEP3 for Windows.[27] Intermolecular interactions were drawn using Mercury 1.4.2 (Build 2).[28] Crystals had good quality and diffracting power, presenting low R_{int} (0.0416 for **6**, 0.0991 for **11**, and 0.0330 for **12**) values that allowed obtaining low *R* values ($R_{1\,all}$ = 0.0401 and $R_{1\,obs}$ = 0.0279 for **6**; $R_{1\,all}$ = 0.0845 and $R_{1\,obs.}$ = 0.0423 for **11**; $R_{1\,all}$ = 0.0324 and $R_{1\,obs.}$ = 0.0273 for **12**) after refining. Relevant details of the X-ray data analysis are presented as Supporting Information.

Biology. Experiments with Human Tumor Cell Lines. The 2-benzoyl-3-ferrocenylbenzo[*b*]thiophene derivatives were dissolved in DMSO just before the experiment, and a calculated amount of drug solution was added to the growth medium, containing cells to a final solvent concentration of 0.5%. This had no discernible effect on cell killing. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], cisplatin, and doxorubicin were obtained from Sigma Chemical Co., St. Louis, MO.

Cell Cultures. Human lung (A549), cervical (A431), and colon (HCT-15) carcinoma cell lines were obtained from ATCC, Rockville, MD. The 2008 cell line, as well as its cisplatin-resistant (C13*) and -revertant (RH4) variants, are human ovarian cancer cell lines and were kindly provided by Prof. G. Marverti (Dept. of Biomedical Science, University of Modena, Italy). The MCF-7 human breast cancer cell line and its multidrug-resistant subline derivative (MCF-7 ADR) were kindly provided by Prof. N. Colabuffo (Dept. Chimico-Biologico, University of Bari, Italy). Cell lines were maintained in the

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(23) : Sheldrick G. M., *SADABS*, Program for Empirical Absorption Correction of Area Detectors (Version 2.10); University of Göttingen: Göttingen, Germany, 2004.

(24) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115.

(25) Sheldrick, G. M. *SHELXL-97*, a Computer Program for the Refinement of Crystal Structures; University of Göttingen: Göttingen, Germany, 1997.

(26) Farrugia, L. J. *WinGX* (v1.64.03b). *J. Appl. Crystallogr.* **1999**, *32*, 837.

(27) Farrugia, L. J. *ORTEP-3 for Windows* (v1.076), based on *ORTEP-III* (v1.03) by: Johnson, C. K.; Burnett, M. N. *J. Appl. Crystallogr.* **1997**, *30*, 565.

(28) Bruno, I. J.; Cole, J. C.; Edgington, P. R.; Kessler, M.; MacRae, C. F.; McCabe, P.; Pearson, J.; Taylor, R. *Acta Crystallogr.* **2002**, *B58*, 389.

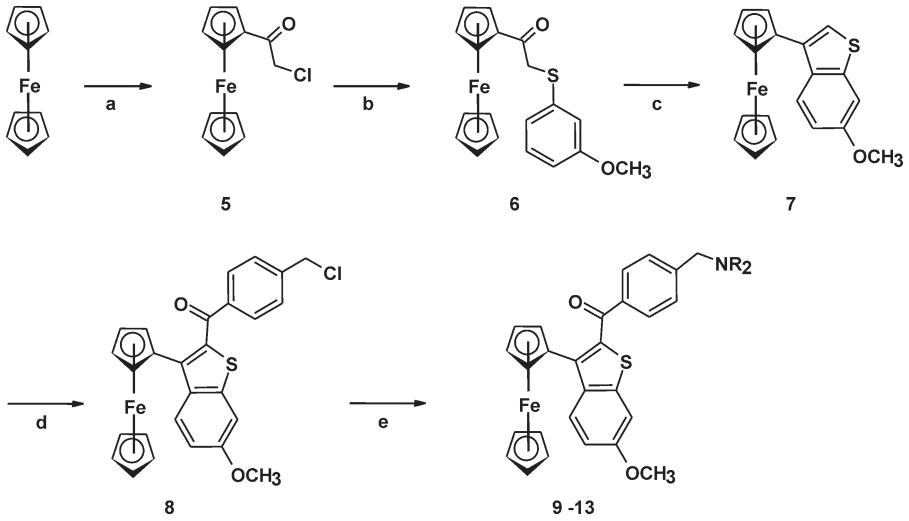


Figure 1. Synthetic sequence leading to the 3-ferrocenyl raloxifene analogues (**9**−**13**) reported in this work. Reaction conditions: (a) $AlCl_3/ClCOCH_2Cl$; (b) *t*-BuOK/3-methoxybenzenethiol; (c) Amberlyst 15; (d) 1. *n*-BuLi; 2. 4-chloromethylbenzoyl chloride; (e) R_2NH. R_2NH = dimethylamine (**9**), piperidine (**10**), pyrrolidine (**11**), morpholine (**12**), and piperazine (**13**).

logarithmic phase at 37 °C in a 5% carbon dioxide atmosphere with the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units·mL^{-1} penicillin and 50 $\mu g \cdot mL^{-1}$ streptomycin), and 2 mM L-glutamine: (i) RPMI-1640 medium (Euroclone) with 25 mM HEPES buffer for HCT-15, MCF-7, MCF-7 ADR, 2008, C13*, RH4, and A431 cells; (ii) D-MEM medium (Euroclone) for A549 cells. The MCF-7 ADR culture medium also contained 0.1 $\mu g \cdot mL^{-1}$ doxorubicin.

Cytotoxicity Assays. The growth inhibitory effect toward tumor cell lines was evaluated by means of the MTT (tetrazolium salt reduction) assay.[29] Briefly, (3−5) × 10^3 cells/well, depending upon the growth characteristics of the cell line, were seeded in 96-well microplates in growth medium (100 μL) and then incubated at 37 °C in a 5% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh one containing the compound to be studied at the appropriate concentration. Triplicate cultures were established for each treatment. After 72 h, each well was treated with 10 μL of a 5 mg·mL^{-1} MTT saline solution, and after 5 h of incubation, 100 μL of a sodium dodecylsulfate (SDS) solution in 0.01 M HCl was added. Following overnight incubation, the inhibition of cell growth induced by the tested derivatives was detected by measuring the absorbance of each well at 570 nm with a Bio-Rad 680 microplate reader. The mean absorbance for each drug dose was expressed as a percentage of the untreated control well absorbance and plotted versus drug concentration. IC_{50} values, which represent the drug concentrations that reduced the mean absorbances at 570 nm to 50% of those in the untreated control wells, were calculated by probit analysis ($P < 0.05$, χ^2 test). Whenever appropriate, resistance factors (RF) were calculated as the ratio of the IC_{50} values obtained for each compound in resistant versus parent cell lines.

Caspase-3 Activity. Caspase-3 activity was detected with the ApoAlert Caspase-3 fluorescent assay kit (Clontech, Mountain View, CA) according to the manufacturer's recommended procedures. In the amount of 10^6, 2008 cells were collected following 12 or 24 h of incubation with the test compounds (at concentrations corresponding to IC_{50} values), lysed on ice for 10 min in 50 μL of lysis buffer, and then treated with 50 μL of reaction buffer containing dithiothreitol (DTT) and 5 μL of caspase-3 substrate solution (Asp-Glu-Val-Asp-7-amino-4-trifluoromethylcoumarin [DEVD-AFC], Clontech). The fluorescence was determined on a Perkin-Elmer 550 spectrofluorometer (excitation 440 nm, emission 505 nm). The caspase-3 activity was expressed as the increase in AFC-emitted fluorescence. Student's *t*-test was used for data analysis.

(29) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.

Results and Discussion

Syntheses and Spectral Characterization. The synthetic sequence toward the 2-acyl-3-ferrocenyl benzo[*b*]thiophenes (**9**−**13**) is outlined in Figure 1. Following a standard Friedel−Crafts-type acylation of ferrocene with chloroacetyl chloride to give **5**, the thioether **6** was obtained by quantitative nucleophilic substitution with 3-methoxybenzenethiolate. The key step in the synthetic sequence was the acid-catalyzed cyclization of **6**, which allowed the construction of the benzo[*b*]thiophene system in a regioselective manner. Presumably, steric constraints determined the predominant ring closure *para* to the methoxy substituent, to give **7**, which was isolated in 72% yield under conventional reflux conditions, as opposed to cyclization *ortho* to both pre-existing substituents in the phenyl ring. In subsequent experiments, the reaction was found to be much faster (20 min versus 4 h) and efficient (>90% yield of the isolated product) when conducted under microwave irradiation at 300 W. Ring closure was readily apparent from the absence of methylene protons in the ^1H NMR spectrum, which were replaced by a one-proton aromatic singlet at 7.41 ppm, assigned to H2 of the benzo[*b*]thiophene. Likewise, the methylene and carbonyl carbons were absent from the ^{13}C NMR spectrum, and two new aromatic carbons, at 133.6 (quaternary, C3) and 118.7 (methine, C2) ppm, were present. The regioselectivity of ring closure was unequivocally established from the presence of a *meta*-coupled doublet in the ^1H NMR spectrum at 7.53 ppm, assigned to H7 of the benzo[*b*]thiophene framework, in addition to an *ortho*-coupled doublet at 8.31 ppm (H4) and a doublet of doublets (J_{ortho} 9.0, J_{meta} 2.4) at 7.15 ppm (H5). Lithiation of **7** was then accomplished at C2 of the benzo[*b*]thiophene ring, and subsequent acylation with chloromethylbenzoyl chloride yielded **8**, which served as the parent compound for a series of derivatives (**9**−**13**) containing tertiary amino substituents.

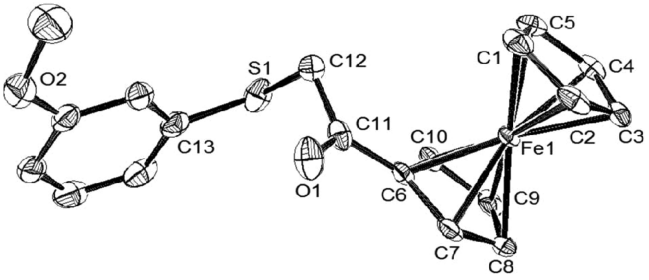


Figure 2. ORTEP diagram, drawn with 50% probability ellipsoids, showing the crystallographic labeling scheme for 1-ferrocenyl-2-[3-(methoxyphenyl)thio]ethanone (**6**).

All compounds described in this work were stable in air. Full characterization was achieved by standard spectroscopic techniques (IR, ^{1}H and ^{13}C NMR, and MS), as well as elemental analyses or HRMS, whenever the compounds retained irreproducible fractional amounts of solvents. All spectral data were fully consistent with the proposed structures. With few exceptions (typically, the methine carbons of the substituted cyclopentadienyl group, for which 3-bond ^{1}H−^{13}C connectivities did not exist) carbon resonances could be assigned from the HSQC and HMBC cross-peak patterns. Prominent characteristic mass spectral fragmentations for compounds **9**−**13** in the ESI mode involved loss of the neutral amines from the protonated molecule, with concomitant formation of a tropylium ion; the loss of cyclopentadiene was also observed in some instances. X-ray quality crystals were grown for the benzothiophenes **11** and **12** and their thioether precursor **6**, which allowed further unequivocal characterization by X-ray diffraction (see below).

Crystal Structures. The structures of 1-ferrocenyl-2-[3-(methoxyphenyl)thio]ethanone (**6**) and of two representative benzo[*b*]thiophenes, [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(pyrrolidin-1-yl)methylphenyl]methanone (**11**) and [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(morpholin-4-yl)methylphenyl]methanone (**12**), were obtained by X-ray diffraction analysis and found to be in agreement with the remaining spectral characterization data. Both compounds **6** and **11** crystallized in the monoclinic $P21/c$ space group, the latter with two crystallographically independent molecules in the asymmetric unit, while compound **12** crystallized in the triclinic $P\bar{1}$ space group. The corresponding ORTEP diagrams are displayed in Figures 2, 3, and 4, respectively. Selected bond lengths and angles for **6**, **11**, and **12** are presented in Table 1.

Both the organometallic moieties and the organic fragments in these three compounds present bond lengths and angles within the expected ranges.[30,31] As anticipated, and due to the relative positioning of the cyclic amino substituents in relation to the ferrocenyl and benzo[*b*]thiophene systems, all the equivalent bonds and angles of molecules **11** and **12** display very similar values.

In all molecules the Cp rings are nearly parallel to each other and their relative conformation is almost fully eclipsed, although molecule **6** shows a larger deviation angle (9.41°) than **11** (2.01°, 1.69°) or **12** (2.16°); this presumably results

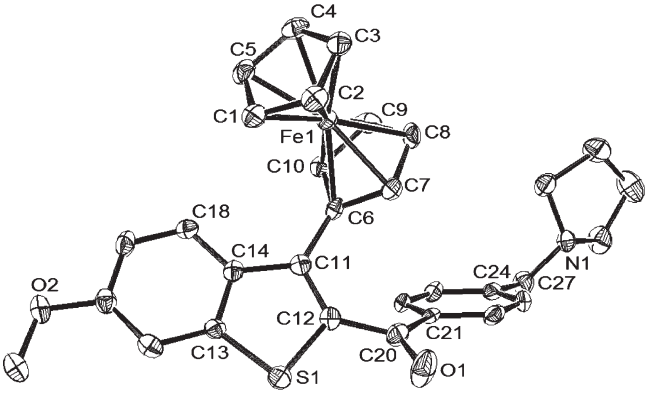


Figure 3. ORTEP diagram, drawn with 50% probability ellipsoids, showing the crystallographic labeling scheme for [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(pyrrolidin-1-yl)-methylphenyl]methanone (**11**, molecule 1).

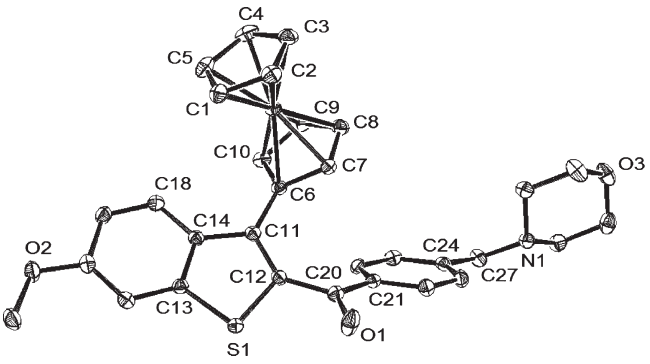


Figure 4. ORTEP diagram, drawn with 50% probability ellipsoids, showing the crystallographic labeling scheme for [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(morpholin-4-yl)-methylphenyl]methanone (**12**).

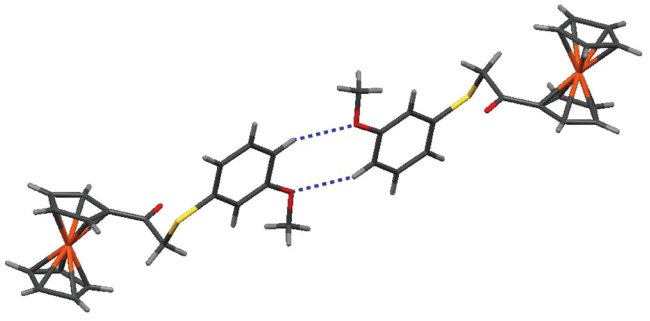


Figure 5. Dimeric arrangement for compound **6**, showing the C−H···O interactions that define a $R_2^2(8)$ synthon.

from a greater mobility of the Cp rings in **6** compared to the benzo[*b*]thiophene derivatives **11** and **12**, where the bulkier groups bound to the ferrocenyl moiety are likely to hinder Cp rotation. The smaller steric hindrance in molecule **6**, and particularly the higher mobility associated with the presence of a sequence of four single bonds (between C6 and C13), may also explain the value for the angle measured between the Cp(C6−10) plane and the phenyl plane (83.57°). The absence of this long sequence of single bonds forces molecules **11** and **12** to display smaller angles (38.32° and 44.06°; 36.84°) between the Cp(C6−10) plane and the closest planar group in the molecule (the fused benzo[*b*]thiophene). It

(30) Orpen, A. G.; Brammer, L.; Allen, F. H.; Kennard, O.; Watson, D. G.; Taylor, R. *J. Chem. Soc., Dalton Trans.* **1989**, S1–S83.
(31) Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1–S19.

Table 1. Selected Bond Lengths and Angles for Compounds 6, 11, and 12

	compound **6**	compound **11** molecule 1	compound **11** molecule 2	compound **12**
		Bonds (Å)		
Fe(1)−Centroid Cp(C1−5)	1.649	1.625	1.632	1.656
Fe(1)−Centroid Cp(C6−C10)	1.640	1.619	1.629	1.653
average Fe−C Cp's	2.040	2.011	2.018	2.050
average C−C Cp's	1.420	1.399	1.399	1.423
C(6)−C(11)	1.467(3)	1.450(5)	1.441(5)	1.4678(18)
C(11)−C(12)	1.507(3)	1.351(5)	1.355(5)	1.3806(19)
C(12)−S(1)	1.820(2)	1.711(4)	1.715(4)	1.7431(15)
S(1)−C(13)	1.776(2)	1.709(4)	1.710(3)	1.7319(16)
C(12)−C(20)		1.457(5)	1.462(5)	1.489(2)
C(20)−C(21)		1.465(5)	1.470(5)	1.4897(19)
C(24)−C(27)		1.490(5)	1.491(5)	1.5127(19)
C(27)−N(1)		1.433(5)	1.427(4)	1.4667(19)
		Angles (deg)		
C(6)−C(11)−C(12)	117.57(19)	121.9(3)	123.3(3)	124.23(12)
C(6)−C(11)−C(14)		126.6(3)	125.9(3)	124.29(11)
C(11)−C(12)−S(1)	111.16(14)	113.9(3)	113.9(3)	112.94(11)
C(12)−S(1)−C(13)	105.05(10)	90.35(18)	90.90(17)	91.62(7)
C(12)−C(20)−C(21)		119.5(3)	119.5(3)	122.93(12)
C(24)−C(27)−N(1)		112.1(3)	111.9(3)	113.45(11)
		Torsion Angles (deg)		
tilt angle[a]	9.41	2.01	1.69	−2.16
		Angles between Planes (deg)		
Cp(C1−C5)−Cp(C6−C10)	2.10	3.05	4.16	3.45
Cp(C6−10)−phenyl	83.57			
Cp(C6−10)−benzothiophene		38.32	44.06	36.84
benzothiophene−phenyl		54.08	59.36	48.27

[a] C(1)−Cp(C1−5)$_{centroid}$−Cp(C6−10)$_{centroid}$−C(6).

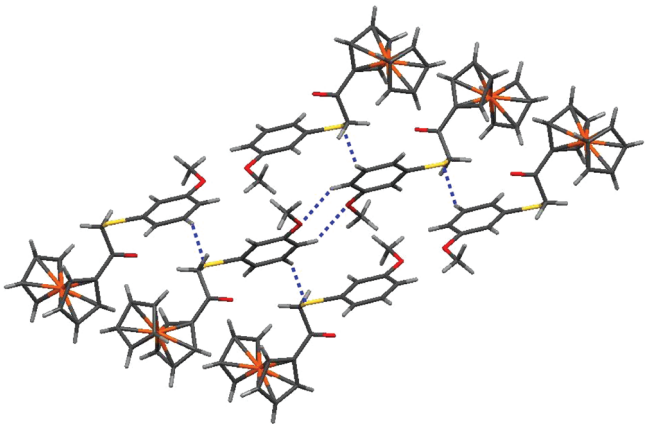


Figure 6. 3D assembly in compound **6** by way of C−H···S interactions.

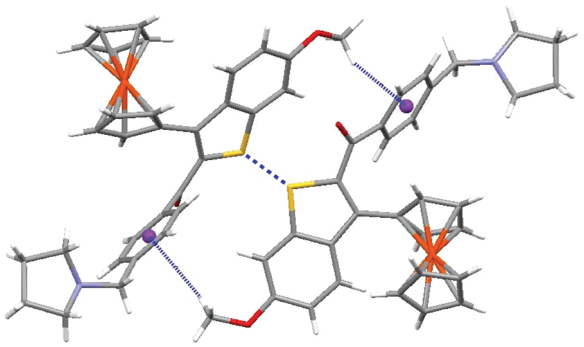


Figure 7. Asymmetric unit of [3-ferrocenyl-6-methoxybenzo-[*b*]thiophen-2-yl][4-(pyrrolidin-1-yl)methylphenyl]methanone (**11**), showing the S···S and C−H$_{methoxy}$···πCp contacts that ensure the dimeric arrangement.

remains to be established if this preferred geometry is a factor in the overall balance of thermodynamic interactions that will determine whether or not compounds **11** and **12** and their analogues are capable of favorable supramolecular interactions with specific biological receptors (e.g., the ERs).

In a preliminary study, the supramolecular array of each molecule was analyzed on the basis of the respective intermolecular interactions. Compound **6** forms dimers through a C−H···O interaction involving the O atom of the methoxy group and a carbon of the phenyl ring, in a $R_2^2(8)$ synthon (distance C···O = 3.461 Å; angle C−H···O = 139.3°; Figure 5); these dimers self-organize in a 3D assembly using C−H···S interactions [distance C−S = 3.778 Å; angle C−H···S = 151.42°; Figure 6 and Figure S1 (Supporting Information)]. The two molecules of the asymmetric unit of compound **11** form a dimer involving S···S (distance S···S = 3.530 Å) and C−H$_{methoxy}$···π_{Cp} contacts (distance C−Cp$_{centroid}$ = 3.500 and 3.577 Å), respectively (Figure 7). These dimers form 2D layers using $\pi-\pi$ interactions between Cp rings (distance = 3.712 Å) aided by C−H$_{pyrrolidine}$···π_{Cp} contacts (distance C−Cp$_{centroid}$ = 3.536 Å) (Figure S2, Supporting Information). Compound **12** also arranges its molecules in dimers, which are based on a $R_2^2(6)$ synthon involving C−H···O methoxy group interactions (distance C···O = 3.215 Å); these dimers self-arrange in a linear

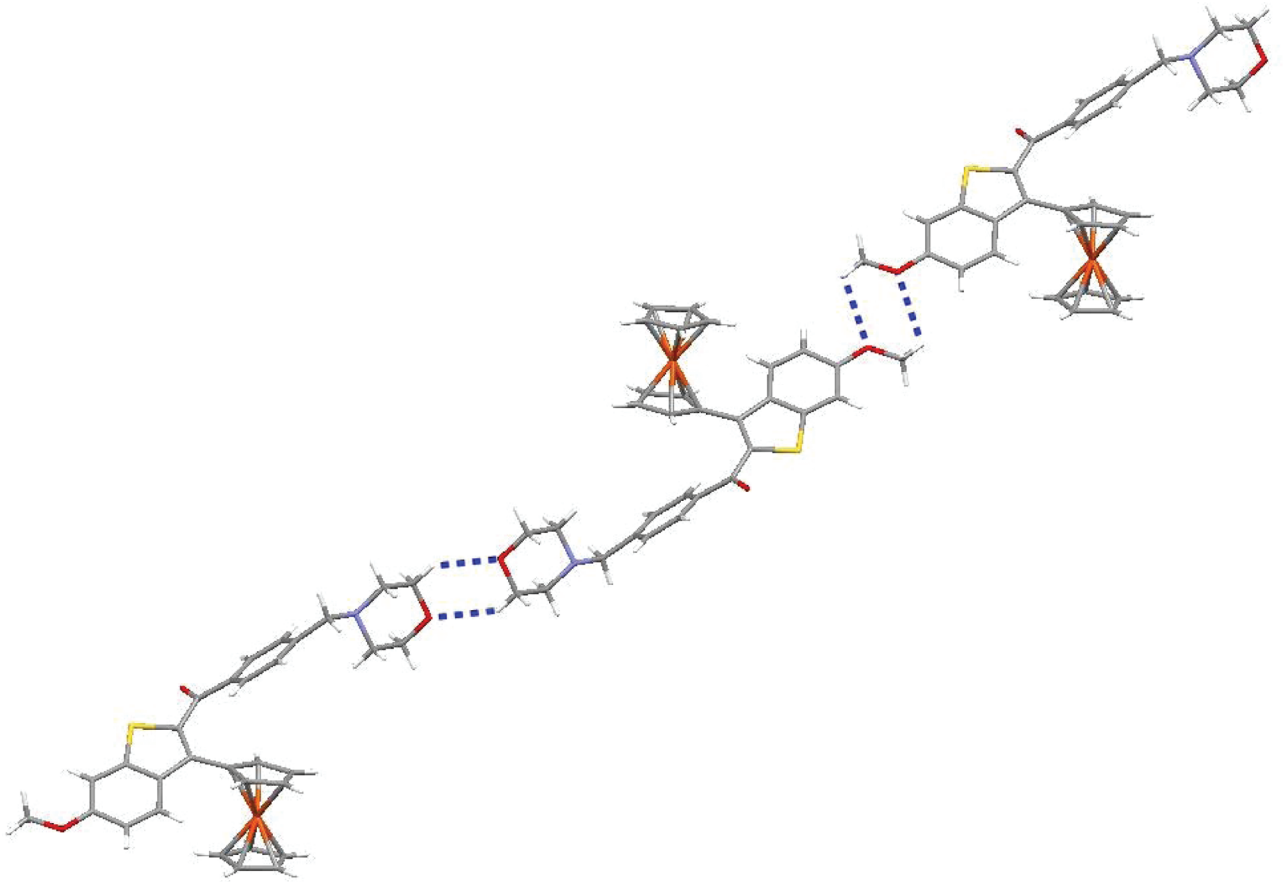


Figure 8. Self-arrangement of [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(morpholin-4-yl)methylphenyl]methanone (**12**), showing the dimer units based on a $R_2^2(6)$ synthon involving C−H···O methoxy group interactions and the assembly of dimers in a linear chain by means of a second $R_2^2(6)$ synthon formed by C−H···O morpholine interactions.

chain by means of another $R_2^2(6)$ synthon formed by C−H···O morpholine interactions (distance C···O = 3.387 Å; angle C−H···O = 144.29°) (Figure 8).

Electrochemical Studies. It is generally accepted that the ferrocene/ferrocenium couple is central to the antitumor activity of ferrocene-based drug molecules. Furthermore, ferrocenium salts have been reported in some instances to be more potent anticancer agents than their neutral counterparts, a property thought to be associated in part with increased oxidative DNA damage stemming from the generation of reactive oxygen species via Fenton chemistry.[32] Representative examples regarding the relevance of the redox properties of ferrocene derivatives to their biological activity are the electrochemical studies conducted by Jaouen and co-workers on a series of ferrocenyl diphenol complexes, which have demonstrated a positive correlation between the ease of oxidation of the ferrocene group and the ensuing cytotoxicity for prostate and breast cancer cell lines.[33] Taking these observations into account, we sought to characterize the redox properties of the ferrocene derivatives synthesized in the current study.

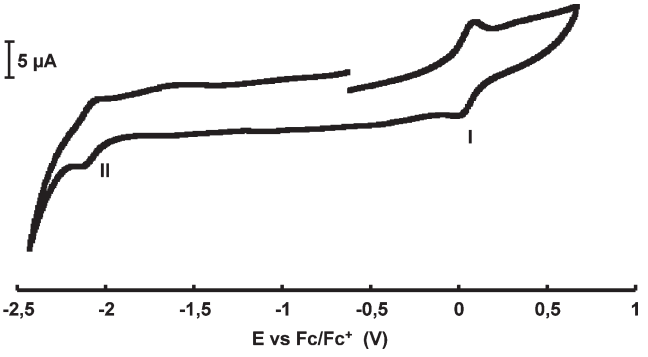


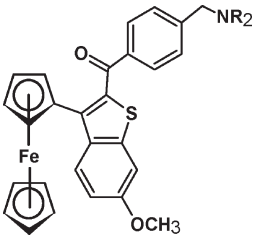
Figure 9. Cyclic voltammogram for [3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperazin-1-yl)methylphenyl]methanone (**13**), in acetonitrile, at a scan rate of 200 mV/s. Waves I and II are discussed in the text.

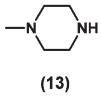
The electrochemical behavior of compounds **9**−**13** was studied by cyclic voltammetry in acetonitrile between the limits imposed by the solvent, i.e., ca. −2.2 and 1.4 V. All measurements were carried out at room temperature, at a scan rate of 200 $mV \cdot s^{-1}$, in a solution containing 0.1 M tetra-*n*-butylammonium hexafluorophosphate as the supporting electrolyte. A representative example of the electrochemical response is shown in Figure 9 for compound **13**, and the most relevant parameters for the redox properties exhibited by the

(32) (a) Köpf-Maier, P. *Z. Naturforsch. C* **1985**, *40*, 843. (b) Neuse, E. W.; Kanzawa, F. *Appl. Organomet. Chem.* **1990**, *4*, 19. (c) Osella, D.; Ferrali, M.; Zanello, P.; Laschi, F.; Fontani, M.; Nervi, C.; Cavigiolio, G. *Inorg. Chim. Acta* **2000**, *306*, 42. (d) Tabbì, G.; Cassino, C.; Cavigiolio, G.; Colangelo, D.; Ghiglia, A.; Viano, I.; Osella, D. *J. Med. Chem.* **2002**, *45*, 5786.

(33) (a) Hillard, E.; Vessières, A.; Thouin, L.; Jaouen, G.; Amatore, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 285. (b) Hillard, E.; Vessières, A.; Le Bideau, F.; Plażuk, D.; Spera, D.; Huché, M.; Jaouen, G. *ChemMedChem* **2006**, *1*, 551. (c) Hillard, E. A.; Pigeon, P.; Vessières, A.; Amatore, C.; Jaouen, G. *Dalton Trans.* **2007**, 5073.

Table 2. Electrochemical Data for Compounds 9−13 in Acetonitrile at Room Temperature[a]



NR_2	E_{pc} (V)	E_{pa} (V)	$E_{p/2}$ (V)	E_{pa}- E_{pc} (mV)	I_c/I_a	pK_a[b]
$-N(CH_3)_2$	+ 0.05	+ 0.12	+ 0.09	70	0.9	10.64
(9)	- 2.09	- 2.02	- 2.06	70	1.1	
	+ 0.05	+ 0.12	+ 0.09	70	1.0	11.22
(10)						
	+ 0.08	+ 0.14	+ 0.11	60	0.9	11.27
	- 2.11	-----	-----	-----	-----	
(11)						
	+ 0.04	+ 0.11	+ 0.08	60	1.2	8.36
(12)						
	+ 0.03	+ 0.10	+ 0.07	70	1.0	5.68, 9.82
	- 2.12	- 2.04	- 2.08	75	1.0	
(13)						

[a] The potentials are referenced to the ferrocene/ferrocenium (Fc/Fc^+) pair ($Ep/2 = 0.40$ V versus SCE).[19] [b] pK_a of the parent amine.[34]

compounds under study are summarized in Table 2. The electrochemical behavior of this family of compounds is predominantly characterized by a quasi-reversible redox wave (process I), in the range 0.07 to 0.11 V, with compound **13** showing the lower value, and an irreversible redox process (process II), in the range −2.02 to −2.11 V, mostly apparent for compounds **9**, **11**, and **13**. The quasi-reversible wave (I) is consistent with a one-electron redox process involving the Fe^{II}/Fe^{III} couple; this wave is up to 110 mV more anodic than that observed for ferrocene,[19] presumably as a result of the electron-withdrawing effect of the 2-acyl substituent(s), which offers extended conjugation to the π-electrons of the cyclopentadienyl ring, thereby decreasing the electron density around the iron atom. Although an increased basicity of the amino group[34] appears to correlate with a small anodic shift of the Fe^{II}/Fe^{III} oxidation peak (Table 2), there is no significant effect as expected from the remoteness of the tertiary amine in relation to the metal center. The irreversible cathodic wave (II) can be attributed to redox processes occurring within the acyl benzo[*b*]thiophene moiety, such as the reduction of the carbonyl group[35] or of the benzo[*b*]thiophene skeleton.[36] Anodic oxidation at the tertiary amino group,[37] though also conceivable for compounds **9**−**13**, was not detected in our experiments.

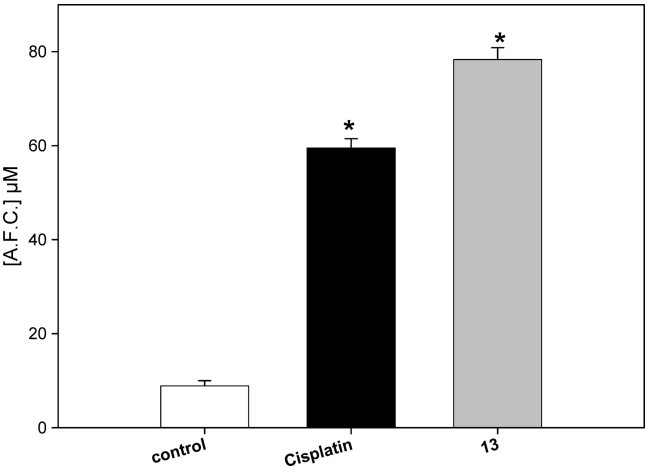


Figure 10. Induction of caspase-3 by 3-ferrocenyl-6-methoxybenzo[*b*]thiophen-2-yl][4-(piperazin-1-yl)methylphenyl]methanone (**13**). Human 2008 ovarian cancer cells were incubated with **13** or cisplatin at the IC_{50} concentration and then submitted to the test on caspase-3 induction, as described in the Experimental Section. The data are the means of at least three independent experiments and express the measured increases in 7-amino-4-trifluoromethylcoumarin (AFC) fluorescence. The error bars represent the standard deviations. The asterisks (*) indicate statistically significant increases ($P < 0.01$) compared to untreated cells.

Cytotoxic Activity. The newly synthesized compounds were evaluated for their cytotoxic activity toward a panel of human tumor cell lines containing examples of ovarian (2008, C13*, RH4), cervical (A431), lung (A549), colon (HCT-15), and breast (MCF-7, MCF-7 ADR) cancers. Cytotoxicity was evaluated by means of MTT tests after 72 h of treatment with increasing concentrations of the test compounds. For comparison purposes, the cytotoxicity of cisplatin was evaluated in the same experimental conditions. The IC_{50} values, calculated from dose−survival curves, are shown in Table 3. All organometallic benzo[*b*]thiophene derivatives showed a marked inhibitory potency toward the different types of tumor cells investigated.

Compound **12**, containing a morpholine substituent, was the least potent but still showed detectable cytotoxic activity; this activity was slightly lower than that of the reference drug, cisplatin, with the mean IC_{50} (μM) values across the tested cell lines being 11.75 (9.35−15.27) and 8.76 (2.56−18.13, excluding the C13* IC_{50} value) for **12** and cisplatin, respectively. Compounds **9**, **10**, and **11**, containing dimethylamine, piperidine, and pyrrolidine substituents, respectively, showed quite similar patterns of response across the various cancer cell lines. A comparison of the mean IC_{50} (μM) values for **9**, **10**, and **11** [5.28 (2.15−7.13), 5.46 (3.01−7.43), and 4.44 (2.27−5.41), respectively] to that of cisplatin (8.76, excluding the C13* IC_{50} value) is indicative of an *in vitro* antitumor activity roughly 2-fold higher than of cisplatin itself.

Among all 2-benzoyl-3-ferrocenylbenzo[*b*]thiophene compounds tested, the piperazinyl derivative (**13**) showed the greatest *in vitro* antitumor efficacy, about 10-fold higher

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Table 3. Cytotoxicity Studies

	IC_{50} (μM) ± SD[a]							
compound	MCF-7	MCF-7 ADR	A431	HCT-15	A549	2008	C13*	RH4
9	5.21 ± 1.11	4.53 ± 1.37 (0.86)[b]	7.13 ± 1.46	6.67 ± 0.91	6.21 ± 1.11	5.52 ± 0.13	4.85 ± 1.54 (0.87)	2.15 ± 0.33
10	5.01 ± 0.96	4.21 ± 1.35 (0.84)	3.01 ± 0.74	5.82 ± 1.62	6.98 ± 0.84	6.32 ± 1.06	4.91 ± 1.04 (1.28)	7.43 ± 1.28
11	4.12 ± 0.23	5.35 ± 0.67 (1.29)	2.27 ± 0.96	3.30 ± 0.68	4.45 ± 0.73	5.29 ± 1.57	5.41 ± 0.96 (1.02)	5.01 ± 1.49
12	11.64 ± 1.28	10.17 ± 1.24 (0.87)	9.71 ± 1.02	10.43 ± 2.12	15.27 ± 1.84	14.43 ± 2.12	9.35 ± 1.23 (0.67)	12.65 ± 2.32
13	1.09 ± 0.79	1.04 ± 0.41 (0.95)	1.01 ± 0.46	2.95 ± 0.95	3.85 ± 1.09	1.26 ± 1.21	1.22 ± 1.06 (0.96)	0.73 ± 0.39
cisplatin	10.32 ± 1.21	8.41 ± 1.43 (0.81)	3.21 ± 1.72	12.31 ± 1.26	18.13 ± 1.51	2.56 ± 1.13	39.21 ± 1.92 (15.31)	6.43 ± 0.68

[a] IC_{50} values toward the indicated cell lines were calculated by probit analysis ($P < 0.05$, χ^2 test). Cells ($(5-8) \times 10^4$ mL^{-1}) were treated for 72 h with increasing concentrations of the test compounds. Cytotoxicity was assessed by the MTT test. SD = standard deviation. [b] The numbers in parentheses are the RF (resistance factor) values, calculated as [IC_{50} (resistant cell line)/IC_{50} (parent cell line)]. The RF for doxorubicin in the multidrug-resistant MCF-7 ADR cell line was 30.75.

than that of cisplatin and with a mean IC_{50} (μM) of 1.64 (0.73−3.85). Moreover, it is noteworthy that the growth inhibitory activity displayed by **13** was more marked against breast, cervical, and ovarian cancer cells (Table 3), which are all particularly sensitive to estrogenic/antiestrogenic effects.[38]

With all benzo[*b*]thiophenes, very interesting results were obtained against cisplatin-resistant (C13*) and -revertant (RH4) cells, as well as against multidrug-resistant (MCF-7 ADR) phenotypes. In ovarian carcinoma C13* cells, cisplatin resistance has been correlated with reduced cell drug uptake, high cellular thioredoxin reductase and glutathione levels, and enhanced repair of DNA damage.[39] From the tests on the 2008/C13* cell pair, the estimated resistance factor of all benzo[*b*]thiophene derivatives **9**−**13** was found to be about 15-fold lower than that of cisplatin (Table 3), clearly revealing no cross-resistance phenomena. RH4 revertant cells were obtained from C13* cells by selection with the lipophilic rhodamine 123 (Rh123) cation. Compared to C13* cells, the RH4 cells lost a substantial portion of their cisplatin resistance, maintaining only a 2- to 3-fold resistance. Despite this major loss of resistance, they retained a number of the phenotypic features related to cisplatin resistance that are observed in C13* cells.[40] As a whole, the behavior of the benzo[*b*]thiophenes **9**−**13** against RH4 cisplatin-revertant cells was comparable to that elicited in the cisplatin-sensitive 2008 cell line (Table 3), which further emphasizes the lack of cross-resistance properties in the new compounds.

Acquired multidrug resitance (MDR), whereby cells become refractory to multiple drugs, poses a most important challenge to the success of anticancer chemotherapy. MCF-7 ADR cells are resistant to doxorubicin, a drug belonging to the MDR spectrum, and this property has been associated with overexpression of multispecific drug transporters.[41] Cytotoxicity assays testing all the benzo[*b*]thiophene derivatives **9**−**13** against the MCF-7/MCF-7 ADR cell line pair showed a similar pattern of response across the parental and resistant subline (Table 3) and allowed the calculation of RF values (0.84−1.29) about 30-fold lower than that obtained with doxorubicin (RF = 30.75), suggesting that these new organometallic benzo[*b*]thiophenes are not potential MDR substrates.

To characterize the cell death pathway triggered by the most active derivative, **13**, we examined its effect in terms of apoptosis induction. Apoptosis is generally considered as one of the main mechanisms of the antitumor effect of cisplatin. Caspase-3 is a well-known executor enzyme in the apoptosis pathway, and cisplatin may cause apoptosis associated with increased caspase-3 activity.[42] Compound **13** was tested for its ability to activate caspase-3 in 2008 human ovarian cancer cells. As shown in Figure 10, a 24 h treatment using IC_{50} concentrations resulted in a significant enhancement of enzyme activity, about 10- and 1.3-fold higher than those detected in untreated and cisplatin-treated cells, respectively. Albeit preliminary, these results, which are consistent with the cytotoxicity data, are encouraging because it is particularly advantageous to develop new agents capable of inducing tumor cell death by means of an apoptotic mechanism, as opposed to a necrotic one.

Conclusions

We have synthesized and characterized a series of 2-benzoyl-3-ferrocenylbenzo[*b*]thiophenes (**9**−**13**) bearing tertiary alkylamino groups linked to the benzoyl C4 through a methylene spacer. These are the first representatives of a class of ferrocenylbenzo[*b*]thiophenes designed as part of a program intended to explore the antitumor and antiestrogenic potential of organometallic compounds having a structural resemblance to the SERM raloxifene. Although the new benzo[b]thiophenes **9**−**13** display a 2-acyl-3-ferrocenyl substitution pattern, as opposed to the 2-aryl-3-acyl substitution of raloxifene, they all showed cytotoxic activity in the low micromolar range toward a series of human cancer cell lines, including cisplatin-resistant (C13*) and -revertant (RH4) human ovarian cells and multidrug-resistant (MCF-7 ADR) breast cancer cells. Of particular interest is the piperazinyl derivative **13**, with a mean IC_{50} about 10-fold lower than that of cisplatin across the tested cell lines. Although potential SERM properties have yet to be evaluated, it is noteworthy that the highest growth inhibitory activities displayed by compound **13** were measured against human gynecological cancer cells (breast, cervix, ovary), which are well known to be sensitive to estrogenic/antiestrogenic effects. Finally, it is also worth mentioning that preliminary caspase-3 induction tests indicated a significant ability of compound **13** to induce cell death by way of an apoptotic mechanism. Taken together, these *in vitro* data suggest that

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the new ferrocenylbenzo[*b*]thiophene derivatives, and compound **13** in particular, may have good antitumor potency. Our observation that the more easily oxidized of the new compounds (**13**) was the one with the lowest mean IC_{50} value is in line with the findings of Jaouen and co-workers on the cytotoxicity of conjugated and unconjugated ferrocenyl phenols[33] and has encouraged us to increase the number and diversity of 2-acyl-3-ferrocenylbenzo[*b*]thiophenes available for biological screening. We are currently applying the synthetic strategy reported herein to expand the library of members of this structural series by varying the spacer size and type and by including different substituents on the benzo[*b*]thiophene and/or organometallic fragments, toward increased intramolecular electron transfer to the ferrocenyl moiety[33] and H-bond donation,[43] which may be at the core of the higher activity observed for the piperazinyl derivative **13**.

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Supporting Information Available: Table S1, summarizing the crystal data and structure refinement parameters for compounds **6**, **11**, and **12**; crystallographic data of the same compounds in CIF format; Figure S1, showing the 3D supramolecular assembly of compound **6** along the *c* axis; Figure S2, showing the 2D layers formed by dimers of compound **11**. These materials are available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for compounds **6**, **11**, and **12** are also deposited with the Cambridge Crystallographic Data Centre (CCDC 692699, 692700, and 692701, respectively) and can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk/deposit).

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