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Synthesis of new bisaryl cyclopentathiophene and thieno-cyclopentoxazolidine derivatives as potential cytotoxic agents

ZIAD OMRAN, PATRICK DALLEMAGNE, & SYLVAIN RAULT

Centre d'Etudes et de Recherche sur le Médicament de Normandie UFR des Sciences Pharmaceutiques Université de Caen Basse Normandie, 5 rue Vaubénard, 14032 Caen cedex France

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Abstract
The synthesis of new bisaryl thienocyclopentoxazolidine derivatives was achieved through a Suzuki cross-coupling procedure with the aim to enhance the previously reported cytotoxicity of the series. The biological activity, evaluated in the NCI's in vitro human disease-oriented tumor cell line screening panel, was however partially conserved by the pharmacomodulations.

Keywords: *Thiophene, cyclopentane, oxazolidine, Suzuki cross-coupling, cytotoxicity*

Introduction

We have previously reported the synthesis and potent cytotoxicity measured in the NCI's in vitro human disease-oriented tumor cell line screening panel of some cyclopenta[c]thiophene derivatives like compounds **1** and **3**, especially against leukaemia cell lines (Figure 1)[1–5]. Some of them exerted further an in vivo antitumor activity assessed in the hollow fiber assay and standard xenograft testing developed by the NCI[6]. In a view to enhance this activity, we have performed further pharmacomodulations in the series and herein we report the study of the replacement of the thiophene bromine atoms of **1,3** by various aromatic rings, using Suzuki cross-coupling.

Materials and methods

Chemistry

Instrumentation. Melting points were determined on a Kofler melting point apparatus and are uncorrected. IR spectra were recorded on a Genesis series FTIR spectrometer using KBr pellets. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Jeol Lambda 400 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants are in Hertz. Electron impact mass spectra (EIMS) were obtained using a Jeol JMS GCMate spectrometer. Reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm Polygram Sil silica gel G/UV 254 precoated plates with visualization by irradiation with a short-wavelength UV light. Silica gel chromatography was performed using 63–200 mM Kieselgel Merck 60 silica gel.

Synthesis

General procedure for the Suzuki cross-coupling reaction. To a solution of 1 mmol of the starting material (**3** or **8**) in DMF (15 mL), boronic acid (4 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium (160 mg, 0.2 mmol) and triethylamine (2mL, 15 mmol) were successively added under argon atmosphere. The mixture was stirred at 65°C for 12 h. 150 mL of distilled water and 30 mL of ethyl acetate were then added. The organic layer was

Correspondence: P. Dallemagne, Centre d'Etudes et de Recherche sur le Médicament de Normandie UFR des Sciences Pharmaceutiques Université de Caen Basse Normandie, 5 rue Vaubénard, 14032 Caen cedex France. Tel: 332 315 65910. Fax: 332 319 33473. E-mail: patrick.dallemagne@unicaen.fr

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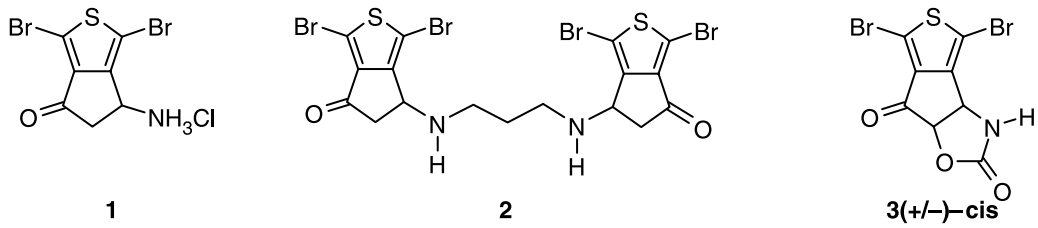


Figure 1. Structure of cytotoxic cyclopentathiophene derivatives **1–3**.

separated, washed three times with distilled water and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The crude bisaryl compounds thus obtained were purified by column chromatography on silica gel (CH_2Cl_2/MeOH 99:1).

*(+/−) 2,2,2-trifluoro-*N*-(6-oxo-1,3-diphenyl-5,6-dihydro-4H-cyclopenta[c]thien-4-yl) acetamide (**12**)*. Yield: 10%. Mp >230°C. IR (cm^{-1}): 3270 (NH), 1698 (CO), 3100, 1560, 1208, 1179, 697. ^{1}H NMR (DMSO-d_6) δ: 9.95 (d, J = 8.5 Hz, 1H, NH), 8.01 (d, J = 7.4 Hz, 2H, H-2′ and H-6′), 7.45 (m, 8H, H-aromatics), 5.37 (dt, J = 3.5, 8.5 Hz, 1H, H-4), 3.55 (dd, J = 8.5, 18.3 Hz, 1H, H-5b), 2.82 (dd, J = 3.5, 18.3 Hz, 1H, H-5a). ^{13}C NMR (DMSO-d_6) δ: 193.90, 155.50 (q, J = 36.2 Hz), 148.51, 143.02, 138.97, 135.01, 131.10, 131.06, 129.60, 128.90, 128.86, 128.35, 127.59, 126.86, 115.53 (q, J = 287.0 Hz), 50.80, 43.75. MS (EI^+) m/z: 401.4 (M^+).

*(+/−) 2,2,2-trifluoro-*N*-(6-oxo-1,3-di-2-thienyl-5,6-dihydro-4H-cyclopenta[c]thien-4-yl) acetamide (**13**)*. Yield: 35%. Mp >230°C. IR (cm^{-1}): 3283 (NH), 1698 (CO), 3099, 1550, 1207, 1180, 695. ^{1}H NMR (DMSO-d_6) δ: 10.10 (d, J = 8.3 Hz, 1H, NH), 8.01 (d, J = 4.2 Hz, 1H, H-3′), 7.75 (d, J = 4.2 Hz, 1H, H-5′), 7.69 (d, J = 4.3 Hz, 1H, H-5″), 7.29 (d, J = 4.3 Hz, 1H, H-3″), 7.20 (t, J = 4.2 Hz, 1H, H-4′), 7.14 (t, J = 4.3 Hz, 1H, H-4″), 5.56 (dt, J = 2.2, 8.3 Hz, 1H, H-4), 3.62 (dd, J = 8.3, 19.0 Hz, 1H, H-5b), 2.80 (dd, J = 2.2, 19.0 Hz, 1H, H-5a). ^{13}C NMR (DMSO-d_6) δ: 193.88, 155.85 (q, J = 37.0), 147.01, 138.03, 134.84, 132.91, 132.44, 129.47, 129.15, 128.70, 128.34, 127.83, 127.51, 126.16, 118.98 (q, J = 286.3 Hz), 51.27, 43.96. HRMS: calculated (412.9831); found (412,9826).

*(+/−) 2,2,2-trifluoro-*N*-(6-oxo-1,3-di-2-furyl-5,6-dihydro-4H-cyclopenta[c]thien-4-yl) acetamide (**14**)*. Yield: 8%. Mp >230°C. IR (cm^{-1}): 3300 (NH), 1700 (CO), 3090, 1550, 1200, 1180, 695. ^{1}H NMR (DMSO-d_6) δ: 10.07 (d, J = 8.3 Hz, 1H, NH), 7.88 (d, J = 2.4 Hz, 1H, H-5′), 7.74 (d, J = 4.3 Hz, 1H, H-5″), 7.65 (d, J = 3.7 Hz, 1H, H-3′), 6.74 (dd, J = 2.4, 3.7 Hz, 1H, H-4′), 6.66 (dd, J = 3.3, 4,3 Hz, 1H, H-4″), 6.61 (d, J = 3.3 Hz, 1H, H-3″), 5.60 (dt, J = 2.4, 8,8 Hz, 1H, H-4), 3.58 (dd, J = 8.8, 18,6 Hz, 1H, H-5b), 3.00 (dd, J = 2.4, 18.6 Hz, 1H, H-5a). ^{13}C NMR (DMSO-d_6) δ: 193.98, 155.55 (q, J = 37.0), 146.61, 140.33, 137.91, 136.44, 132.83, 132.11, 131.40, 131.10, 123.53, 123.32, 121.61, 120.26, 118.99 (q, J = 286.3 Hz), 51.29, 43.09. MS (EI^+) m/z: 381.1 (M^+).

*(+/−) 6-amino-1,3-di-2-thienyl-5,6-dihydro-4H-cyclopenta[c]thiophen-4-one hydro-chloride (**15**)*. A suspension of **13** (200 mg) in a mixture of ethanol (5 mL) and 6N aqueous solution of hydrochloric acid (15 mL) was refluxed for 7 days. The mixture was then evaporated to dryness under reduced pressure. The residue was then thoroughly washed with acetone, filtered and dried to give fluorescing red crystals. Yield: 20%. Mp >230°C. IR (cm^{-1}): 3428 (NH), 1703 (CO), 2922, 1083, 798, 697. ^{1}H NMR (DMSO-d_6) δ: 8.75 (s, 3H, NH_3), 7.60 (m, 6H, H-aromatics), 5.56 (m, 1H, H-6c), 3.62 (m, 1H, H-5b), 2.94 (dd, J = 2.2, 18.9 Hz, 1H, H-5a). ^{13}C NMR (DMSO-d_6) δ: 192.47, 144.59, 137.39, 135.77, 133.71, 132.67, 131.93, 129.87, 129.45, 128.96, 128.77, 127.96, 127.17, 49.01, 43.82. MS (EI^+) m/z: 381.1 (M^+ -HCl).

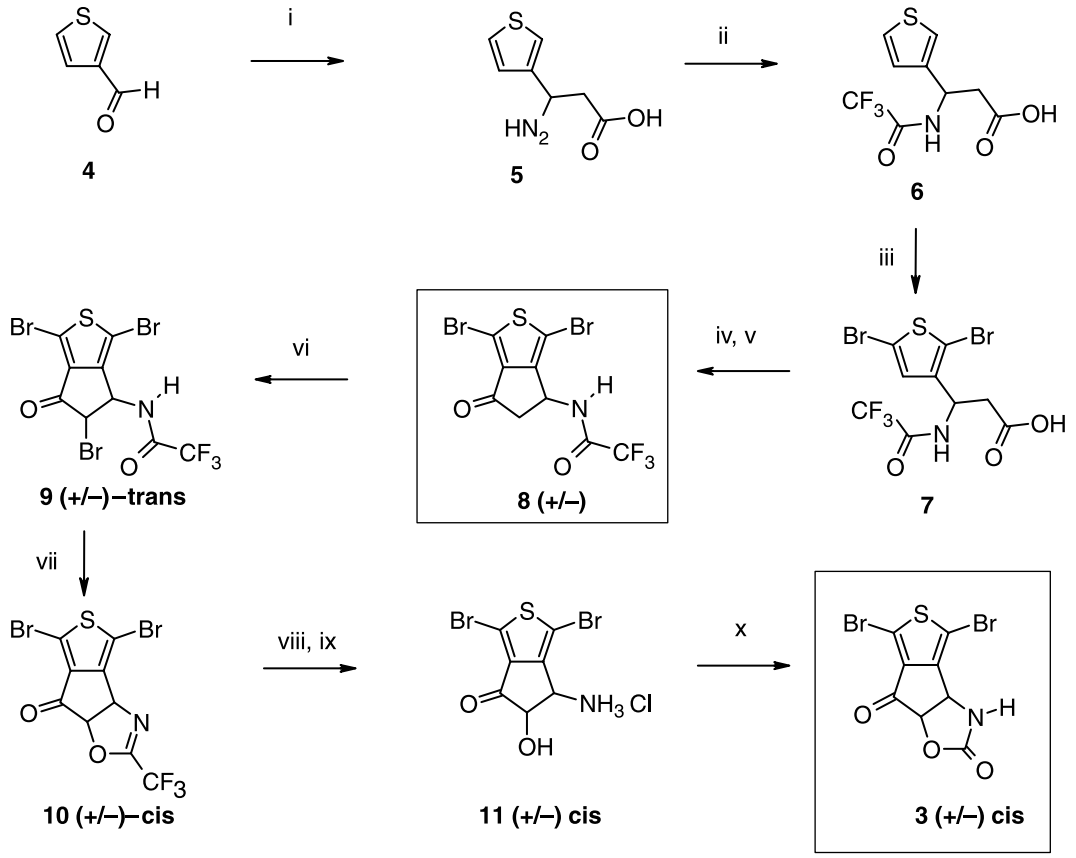
*(+/−)-cis 4,6-dithien-2-yl-3a,7a-dihydro-2H-thieno[3′,4′:4,5]cyclopenta[1,2-d][1,3] oxazole-2,7(3H)-dione (**16**)*. Yield: 35%. Mp >230°C. IR (cm^{-1}): 3433 (NH), 1755 (CO), 1702 (CO), 2924, 1480, 1261, 1091, 697. ^{1}H NMR (DMSO-d_6) δ: 8.84 (laberge, 1H, NH), 8.01 (d, J = 3.7 Hz, 1H, H-3′), 7.80 (d, J = 4.9 Hz, 1H, H-5″), 7.73 (d, J = 4.9 Hz, 1H, H-3″), 7.49 (d, J = 3.7 Hz, 1H, H-5′), 7.22 (t, J = 4,9 Hz, 1H, H-4″), 7.19 (t, J = 3.7 Hz, 1H, H-4′), 5.45 (d, J = 7.4 Hz, 1H, H-3a), 5.34 (d, J = 7.4 Hz, 1H, H-7a). ^{13}C NMR (DMSO-d_6) δ: 189.86, 157.58, 147.02, 138.60, 134.60, 132.75, 132.16, 130.29, 129.76, 128.88, 128.80, 128.47, 127.68, 126.61, 83.66, 50.35. HRMS: calculated (358.9744), found (358.9734).

*(+/−)-cis 4,6-dithien-3-yl-3a,7a-dihydro-2H-thieno[3′,4′:4,5]cyclopenta[1,2-d][1,3] oxazole-2,7(3H)-dione (**17**)*. Yield: 31%. Mp >230°C. IR (cm^{-1}): 3420 (NH), 1755 (CO), 1693 (CO), 2920, 1490, 1342, 1259, 1064, 797. ^{1}H NMR (DMSO-d_6) δ: 8.92 (laberge, 1H, NH), 8.62 (d, J = 2.9 Hz, 1H, H-2′), 7.98 (d, J = 2.8 Hz, 1H, H-2″), 7.83 (dd, J = 2.8, 5.1 Hz, 1H, H-4″), 7.80 (dd, J = 2.9, 5.1 Hz, 1H, H-4′), 7.75 (d, J = 5.1 Hz, 1H, H-5′), 7.52

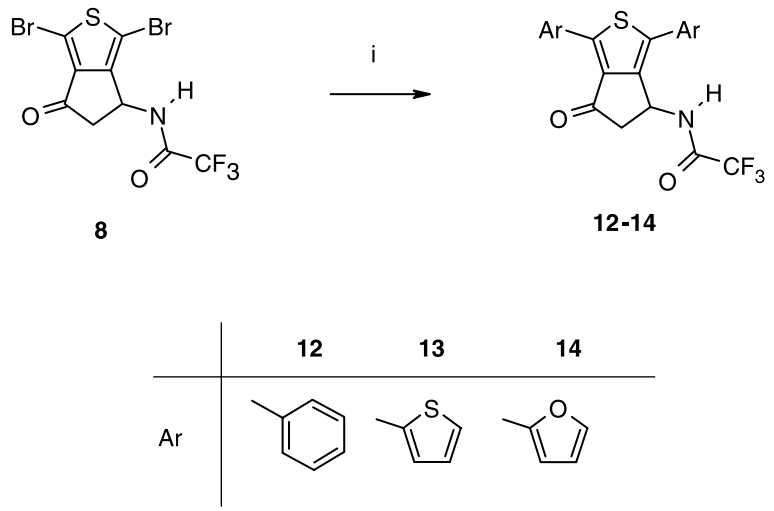
(d, J = 5.1 Hz, 1H, H-5″), 5.53 (d, J = 7.6 Hz, 1H, H-3a), 5.47 (d, = 7.6 Hz, 1H, H-7a). ^{13}C NMR (DMSO-d_6) δ: 190.66, 157.92, 147.19, 140.62, 135.06, 132.17, 131.41, 130.60, 128.48, 128.39, 127.09, 126.90, 126.65, 123.61, 83.93, 50.47. HRMS: calculated (358.9744), found (358.9740).

*(+/−)-cis 4,6-bis(5-bromo-2-thienyl)-3a,7a-dihydro-2H-thieno[3′,4′:4,5]cyclopenta[1,2-d] [1,3]oxazole-2,7(3H)-dione (**18**)*. A solution of **16** (360 mg, 1 mmol) in dichoromethane (20 mL) was treated with 1 M solution of bromine in dichloromethane (2.2 mL, 2.2 mmol) at room temperature. The reaction mixture was refluxed for 30 min. and then quenched with 5% $Na_2S_2O_5$ aqueous solution. The organic layer was separated, washed twice with brine, dried over $CaCl_2$ and filtered. The solvent was removed under reduced pressure. The compound so obtained was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 99:1). Yield: 85%. Mp >230°C. IR (cm^{-1}): 3435 (NH), 1752 (CO), 1709 (CO), 2925, 1579, 1425, 1096, 796. ^{1}H NMR (DMSO-d_6) δ: 8.83 (laberge, 1H, NH), 7.72 (d, J = 4.0 Hz, 1H, H-3′), 7.34 (d, J = 4.0 Hz, 1H, H-4′), 7.32 (d, J = 3.9 Hz, 1H, H-3″), 7.29 (d, J = 3.9 Hz, 1H, H-4″), 5.43 (d, J = 7.8 Hz, 1H, H-3a), 5.31 (d, J = 7.8 Hz, 1H, H-7a). ^{13}C NMR (DMSO-d_6) δ: 190.00, 157.70, 147.94, 137.53, 135.23, 134.34, 133.84, 132.21, 132.18, 130.34, 127.51, 127.43, 116.08, 113.04, 83.53, 50.20. MS (EI^+) m/z: 514.7 (M^+-2), 516.7 (M^+), 518.7 (M^+ + 2).

*(+/−)-cis 4,6-bis(2,5-dibromo-3-thienyl)-3a,7a-dihydro-2H-thieno[3′,4′:4,5]cyclopenta [1,2-d][1,3]oxazole-2,7(3H)-dione (**19**)*. A solution of **17** (360 mg, 1 mmol) in dichoromethane (20 mL) was treated with 1 M solution of bromine in dichloromethane (4.4 mL, 4.4 mmol) at room temperature. The reaction mixture was refluxed for 45 min., and then quenched with 5% $Na_2S_2O_5$ aqueous solution. The organic layer was separated, washed twice with brine, dried over $CaCl_2$ and filtered. The solvent was removed under reduced pressure. The compound thus obtained was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 98:2). Yield: 77%. Mp >230°C. IR (cm^{-1}): 3413 (NH), 1790 (CO), 1718 (CO), 2923, 1472, 1360, 1080, 804. ^{1}H NMR (DMSO-d_6) δ: 8.82 (laberge, 1H, NH), 7.78 (s, 1H, H-4′), 7.59 (s, 1H, H-4″), 5.40 (m, 2H, H-3a and H-7a). ^{13}C NMR (DMSO-d_6) δ: 189.81, 157.37, 150.45, 137.62, 136.52, 132.58, 131.87, 131.81, 131.38, 128.82, 112.50, 112.27, 111.27, 110.76, 83.49, 49.82. MS (EI^+) m/z: 670.9 (M^+-4), 672.9



Scheme 1. Synthesis of compounds **3,8**. Reagents: (i) $AcONH_4$, $(CH_2)_2CO_2H$, EtOH; (ii) TFA_2O, Et_2O; (iii) Br_2, CH_2Cl_2; (iv) $SOCl_2$; (v) $AlCl_3$, CH_2Cl_2; (vi) Br_2, AcOH; (vii) Na_2CO_3, Me_2CO; (viii) HCl gas, Me_2CO; (ix) ambient air; (x) $(CCl_3O)_2CO$, toluene.



Scheme 2. Synthesis of compounds **12–14**. Reagents: (i) $ArB(OH)_2$, $PdCl_2(dppf)$, TEA, DMF.

(M^+-2), 674.9 (M^+), 676.9 ($M^+ + 2$), 678.9 ($M^+ + 4$).

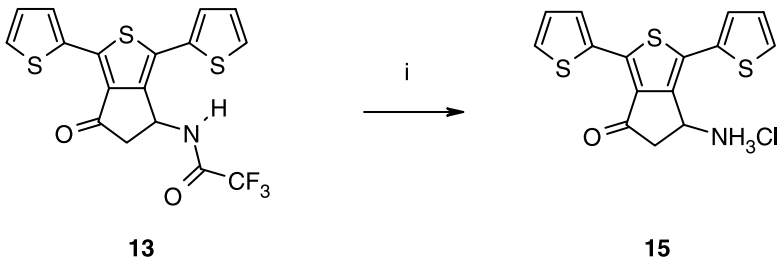
Cytotoxic assays

The cytotoxic activity of tested compounds was evaluated in the NCI's in vitro human disease-oriented tumor cell-line screening panel[7]. The later consists of 60 human tumor cell lines. Nine subpanels represent diverse histologies, *i.e.* nonsmall cell lung, renal, breast cancers, central nervous system, colon, melanoma, prostate, ovarian, and leukemia. The screening is a two-stage process, beginning with the evaluation of compounds against three-cell lines (lung: NCI-H460; breast: MCF7; CNS: SF-268) at a single dose. The results are expressed as the percent growth at 10^{-4} M concentration. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five different concentration levels. Results are evaluated in terms of specificity and potency. The cytotoxic effects of each of these compounds are expressed as the molar drug concentration required for 50% growth inhibition (GI_{50}).

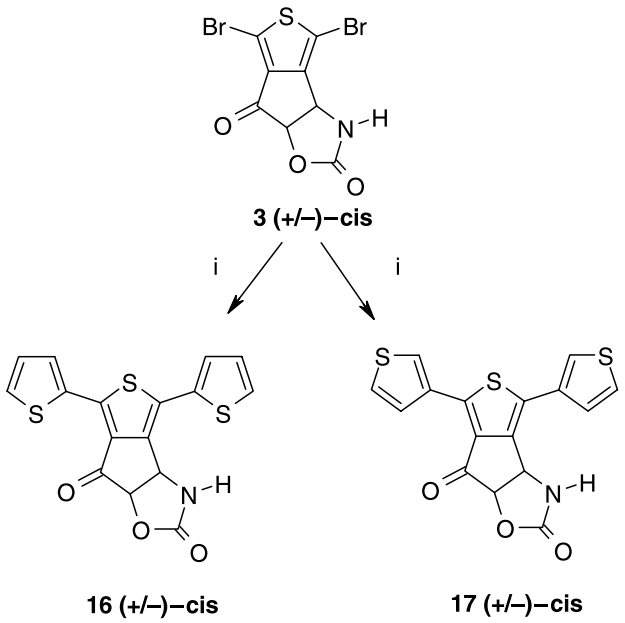
Results and discussion

Chemistry

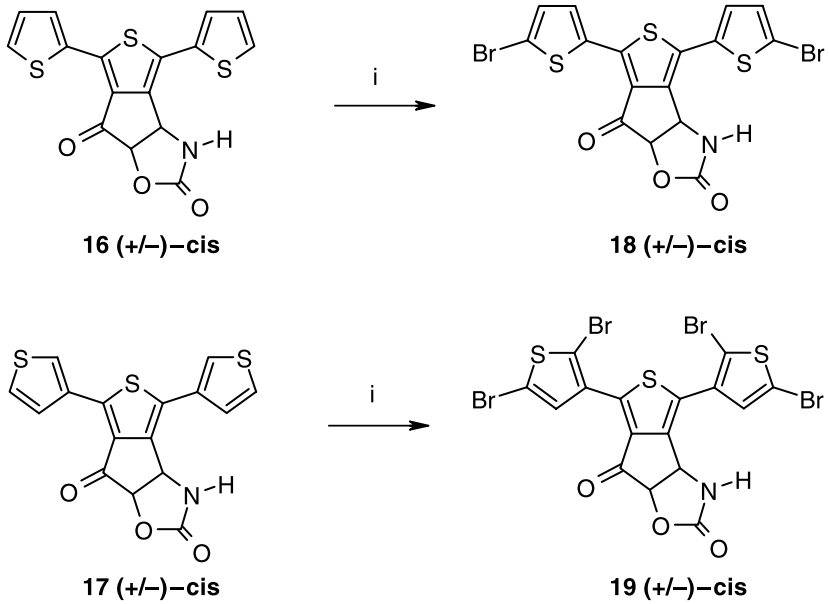
Suzuki cross-couplings were achieved starting from either compound **3** or a N-protected derivative of **1**, the trifluoroacetamide **8** which constitutes an intermediate in the access to **3**.The multi-step synthesis of **8** has been reported by us[1] using thiophene-3-carboxaldehyde **4** as starting material that involved a Rodionov-Johnson reaction to give the β-aryl-β-aminoacid **5** which after N-protection and bromination at the alpha positions of thiophene and Friedel-Crafts cyclisation led to the trifluoroacetylamino-cyclopenta[c]thiophenone **8** as a racemic mixture of (+) and (−) stereoisomers (Scheme 1). Synthesis of **3** was further diastereoselectively achieved through a sequence of steps involving first a selective mono trans bromination of the free alicyclic methylene group followed by ring closure in alkaline conditions that lead to the cis trifluoromethyloxazole derivative **10**; Cleavage of which under acidic conditions retained the cis configuration of the hydroxyl and amino groups (compound **11**). Final treatment of the latter with triphosgene in toluene



Scheme 3. Synthesis of compound **15**. Reagents: (i) HCl 6N.



Scheme 4. Synthesis of compounds **16–17**. Reagents: (i) $ArB(OH)_2$, $PdCl_2(dppf)$, TEA, DMF.



Scheme 5. Synthesis of compounds **18–19**. Reagents: (i) Br_2, CH_2Cl_2.

afforded the attempted racemic cis isomers of thienocyclopentoxazolidinone **3**.

The Suzuki reaction was realized starting from **8** in DMF by treatment with phenyl, 2-thienyl or 2-furylboronic acids (3 or 4 eq) in the presence of TEA and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium as catalyst according to the experimental conditions established in our lab (Scheme 2)[8]. It led to bis aryl cyclopentathiophenones **12–14** respectively with poor yields. Variations of the experimental conditions didn't allow the synthesis of single arylsubstituted derivatives.

As with our observation with polyaromatic cyclopentatrifluoroacetamides, the N-protective group of **12–14** was difficult to cleave, the only deprotection achieved was of **13**, after seven days of hydrochloric acid hydrolysis, to its ammonium salt **15** with 20% yield (Scheme 3).

Similarly oxazolidinone **3** treated with 2- or 3-thienylboronic acids, led to the corresponding bisaryl derivatives **16** and **17** respectively in moderate yield (Scheme 4). In view to attempt to recover the benefit of the bromine atoms in the cytotoxicity, compounds **16** and **17** were further treated with bromine in refluxing dichloromethane to yield the bis monobromo **18** and dibromo **19** derivatives respectively (Scheme 5). All these bisaryl oxazolidinones retained the cis configuration of their starting material **3**.

Table I. Cytotoxicity of compounds **15–19** in the NCI's three-cell line one-dose primary anticancer assay, expressed as the percent growth at 10^{-4} M concentration.

Compd	Lung NCI-H460	Breast MCF7	CNS SF-268
15	19	60	33
16	46	53	70
17	80	74	113
18	112	63	127
19	114	76	128

Table II. Cytotoxicity of compounds **1,3,15** in the NCI's in vitro human disease-oriented tumor cell line screening expressed as the log molar drug concentration required for 50% growth inhibition (log GI_{50})

Compd	Leukemia RPMI 8226	Lung NCI-H522	Colon SW-620	CNS SF-539	Melanoma UACC-257	Ovarian ovcar-4	Renal CAKI-1	Prostate PC-3	Breast MDA-231	**MGM**[a]
1	−6.53	−5.81	−5.50	−5.61	−7.23	−5.71	−5.76	−5.13	−5.68	**−5.46**
3	−5.37	−6.61	−5.68	−5.11	−5.74	−5.78	−6.47	−5.02	−5.74	**−5.53**
15	−4.91	−4.65	−5.04	−4.67	−4.28	−4.37	−4.48	−4.51	−4.55	**−4.61**

[a] Mean Graph Midpoint for all human cancer cell lines tested.

Biology

Compounds **15–19**, synthesized by Suzuki cross-coupling, were first evaluated in the NCI's three-cell line one-dose primary anticancer assay. The results, expressed as percent growth at 10^{-4} M concentration, are summarized in Table I. The cytotoxicity dramatically decreased in this new series since only the ammonium salt **15** showed a weak activity especially against the lung cell-line NCI H-460 (percent growth = 19%), whereas the oxazolidinones, brominated or not, were totally devoid of such activity.

On the basis of this preliminary test, **15** was evaluated in the *in vitro* human disease-oriented tumor cell line screening panel.[6] The log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table II. The MGM is based on a calculation of the average log GI_{50} for all of the cell lines tested (approximately 60) in which GI_{50} values below and above the test range (10^{-4}–10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test.

These results indicated a weak cytotoxicity for **15** with a MGM log GI_{50} value of −4.61, corresponding to a MGM GI_{50} value of 24 μM, *ie* approximatively 8 fold less active than **1** (3.5 μM) and **3** (3 μM). This study confirms again the crucial role played by the dibromothiophene moiety in the cytotoxicity of the cyclopentathiophenone series. The latter however allows further numerous pharmacomodulations which are currently under investigation.

References

[1] Dallemagne P, Alsaïdi A, Boulouard M, Rault S, Robba M. Heterocycles 1993;36:287–294.
[2] Alsaïdi A, Al Shargapi S, Dallemagne P, Carreiras F, Gauduchon P, Rault S, Robba M. Chem Pharm Bull 1994;42:1605–1608.
[3] Dallemagne P, Pham Khanh L, Alsaïdi A, Renault O, Varlet I, Collot V, Bureau R, Rault S. Bioorg Med Chem 2002;10:2185–2191.
[4] Pham Khanh L, Dallemagne P, Landelle HR, Rault S. J Enz Inhib Med Chem 2002;6:439–442.
[5] Dallemagne P, Pham Khanh L, Alsaïdi A, Varlet I, Collot V, Paillet M, Bureau R, Rault S. Bioorg Med Chem 2003;11:1161–1167.
[6] Hollingshead MG, Alley MC, Camalier RF, Abbott BJ, Mayo JG, Malspeis L, Grever MR. Life Sci 1995;57:131–141.
[7] Boyd MR, Paull KD. Drug Dev Res 1995;34:91–109.
[8] Ruhland B, Bombrun A, Gallop MA. J Org Chem 1997;62:7820–7826.