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Synthesis and Biological Evaluation of Thienopyrrolizines, a New Family of CDK/GSK-3 Inhibitors

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Fifteen new thieno[2,3-*b*]- and thieno[3,4-*b*]pyrrolizines were synthesized and tested against two protein kinases, CDK1/cyclin B and GSK-3. Among these compounds, 3-(3-hydroxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one 4g was identified as a moderate inhibitor of these kinases. Its molecular modeling study brought to the fore the pivotal role of the 2-methoxyphenol grouping and the interest in replacing it by bioisosteric moieties in future pharmacomodulations.

Keywords: Thieno[2,3-*b*]pyrrolizines; Thieno[3,4-*b*]pyrrolizines; Protein kinases; CDK1/cyclin B; GSK-3; Phosphorylation

INTRODUCTION

Phosphorylation on serine, threonine and tyrosine residues by protein kinases using ATP or GTP as the phosphate donor, constitutes one of the most common mechanisms of post-translational modifications of proteins. Due to the importance of these phosphorylations in most cellular events, these protein kinases constitute very interesting targets involved in numerous human diseases. Among the human kinases, two classes have been particularly studied: cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3 (GSK-3). Some CDKs are involved in controlling the cell cycle, apoptosis and appeared to be deregulated in many human cancers. Other CDKs are implicated in some neurodegeneration phenomena.[1–7] GSK-3, so-called because it phosphorylates and inactivates glycogen synthase, is also involved in multiple signaling pathways.[8–12] Inhibitors of CDKs and GSK-3 have a promising potential against several diseases such as cancer, diabetes and neurodegenerative disorders (Alzheimer disease).[13,14] Some of them are dual inhibitors of the two classes of enzymes, acting through competition with ATP binding. Among these compounds are paullones **1**,[15] indirubins **2**[16] and phenylpyrrolopyrazines namely aloïsines **3**[17] (Figure 1). Taking into account the potential therapeutic interest of such compounds, the search for new CDKs/GSK-3 inhibitors remains an exciting challenge.

In this paper, we report the identification, among the library of our laboratory, of a series of thienopyrrolizinones **4** and **5** as a new family of CDK1/GSK-3 inhibitors which act in the micromolar range. Molecular modeling of the most active compound **4g** in the active site of GSK-3, in comparison with reference inhibitors, was carried out so as to design new pharmacomodulations likely to enhance the inhibitory activity of the studied series.

MATERIALS AND METHODS

Chemicals

Instrumentation

Melting points were determined on a Kofler melting point apparatus and are uncorrected. Elemental analysis of new compounds was performed at the Institut de Recherche en Chimie Organique Fine (Rouen, France). IR spectra were recorded on a Genesis series FTIR spectrometer using KBr pellets.

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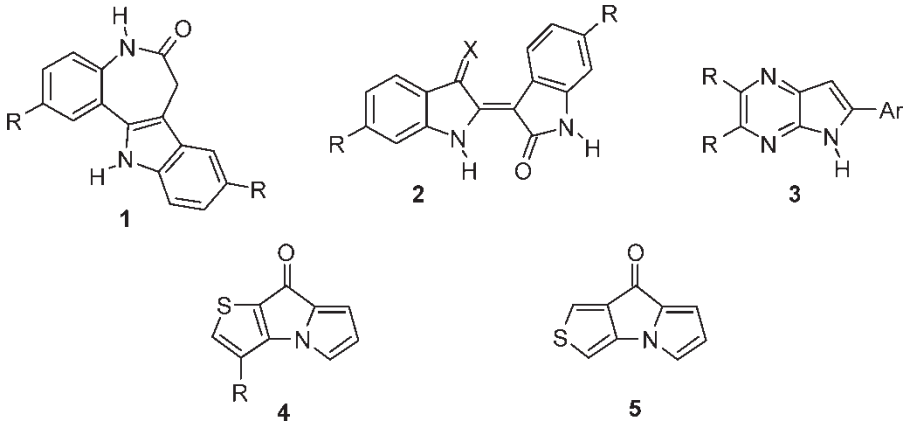


FIGURE 1 Structure of some literature CDKs/GSK-3 inhibitors **1**–**3** and thienopyrrolizines **4**,**5**.

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 short-wavelength UV light. Silica gel flash chromatography was performed using 63–200 mM Kieselgel Merck 60 silica gel.

Synthesis

3-(3,4-Diacetoxyphenyl)-8*H*-thieno[2,3-*b*]-pyrrolizin-8-one (**4h**)

To an ice-cooled solution of **4f** (0.5 g, 0.0018 mol) in freshly distilled THF (50 mL) was added portionwise acetic anhydride (0.34 mL, 0.0036 mol). The reaction mixture was then refluxed for 3 h and evaporated to dryness under reduced pressure. The residue was triturated in iced water (40 mL), then extracted twice by CH_2Cl_2 (2 × 40 mL). The organic layer was separated, washed twice by a saturated aqueous solution of $NaHCO_3$ (2 × 40 mL) and dried over $CaCl_2$. After filtration, the solvent was removed under reduced pressure. The solid residue was recrystallized from Et_2O to give pure **4h** as an orange solid. Yield: 92%. Mp = 190°C. IR (cm^{-1}): 1754 (CO), 1681 (CO), 1524, 1371, 1197, 1112, 1009, 787, 724. ^{1}H NMR (DMSO-d_6) δ: 8.18 (s, 1 H, H2), 7.60 (d, J = 1.7 Hz, 1 H, H2′), 7.53 (dd, J = 8.3, 1.7 Hz, 1 H, H6′), 7.42 (d, J = 8.3 Hz, 1 H, H5′), 6.96 (d, J = 2.2 Hz, 1 H, H5), 6.78 (d, J = 3.3 Hz, 1 H, H7), 6.17 (m, 1 H, H6), 2.32 (s, 3 H, Me), 2.30 (s, 3 H, Me). ^{13}C NMR (DMSO-d_6) δ: 173.7, 168.1, 168.0, 150.5, 142.3, 142.2, 135.8, 134.8, 130.8, 127.7, 127.3, 126.1, 124.2, 123.1, 121.3, 115.9, 113.6, 20.6, 20.5. MS (EI^+) *m*/*z* 367 (M^+, 23.4), 325 (19.3), 283 (100), 244 (31.8), 153 (18.6), 91 (61.7). Found: C, 62.18; H, 3.53; N, 3.97. $C_{19}H_{13}NO_5S$ requires: C, 62.11; H, 3.56; N, 3.81%.

3-(3,4-Diethoxycarbonyloxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (**4i**)

Using the same procedure as for **4h** and starting from a solution of **4f** (0.5 g, 0.0018 mol) in THF (50 mL) and propionic anhydride (0.46 mL, 0.0036 mol), **4i** was obtained as an orange solid. Yield: 83%. Mp = 150°C. IR (cm^{-1}): 3074, 2976, 1769 (CO), 1673 (CO), 1555, 1474, 1371, 1262, 1139, 1050, 751. ^{1}H NMR (DMSO-d_6) δ: 8.17 (s, 1 H, H2), 7.60 (d, J = 1.6 Hz, 1 H, H2′), 7.52 (dd, J = 8.1, 1.6 Hz, 1 H, H6′), 7.42 (d, J = 8.1 Hz, 1 H, H5′), 6.95 (d, J = 2.4 Hz, 1 H, H5), 6.77 (d, J = 3.6 Hz, 1 H, H7), 6.14 (m, 1 H, H6), 2.62 (s, 4 H, 2 CH_2), 1.14 (s, 6 H, 2 Me). ^{13}C NMR (DMSO-d_6) δ: 173.9, 168.5, 168.3, 151.2, 142.4, 142.3, 135.3, 134.5, 131.1, 127.7, 127.6, 126.4, 124.3, 123.7, 121.4, 116.1, 113.7, 35.4, 35.2, 15.7, 15.6. MS (EI^+) *m*/*z* 395 (M^+, 74.1), 339 (65.8), 283 (100), 226 (12.3), 198 (10.9). Found: C, 63.50; H, 4.29; N, 3.51. $C_{21}H_{17}NO_5S$ requires: C, 63.78; H, 4.33; N, 3.54%.

3-(2-Oxo-1,3-benzodioxol-5-yl)-8*H*-thieno[2,3-*b*]-pyrrolizin-8-one **(4j)**

To an 1 M aqueous potassium hydroxide solution (30 mL) was added **4f** (0.5 g, 0.0018 mol). The solution was cooled to 0°C and a 20% solution of phosgene in toluene (10 mL) was added. The reaction mixture was stirred at room temperature for 15 min and then extracted with EtOAc (50 mL). The organic layer was separated, washed twice with water (2 × 50 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was recrystallized from cyclohexane to give pure **4j** as an orange solid. Yield: 84%. Mp = 103°C. IR (cm^{-1}): 1835 (CO), 1680 (CO), 1502, 1482, 1275, 1024, 789, 738. ^{1}H NMR ($CDCl_3$) δ: 7.12 (m, 7 H, H_{arom}). ^{13}C NMR ($CDCl_3$) δ: 174.2, 169.9, 150.8, 142.1, 141.8, 136.0, 134.9, 130.8, 127.5, 127.1, 125.3, 124.3, 123.6, 121.7, 116.4, 113.5. Found: C, 62.12; H, 2.28; N, 4.53. $C_{16}H_7NO_4S$ requires: C, 62.13; H, 2.28; N, 4.53%.

3-(3-Acetoxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (**4k**)

To an ice-cooled solution of **4g** (0.6 g, 0.002 mol) in AcOH (10 mL) was added portion-wise Ac_2O (10 mL). The reaction mixture was then heated at 100°C for 15 h and evaporated to dryness under reduced pressure. The oily residue was triturated in cooled water (50 mL) for 1 h and then extracted with $CHCl_3$ (50 mL). The organic layer was separated, washed twice with a saturated aqueous $NaHCO_3$ solution (2 × 100 mL), dried over $CaCl_2$, filtered and the solvent was then removed under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH: 95/5) to give pure **4k** as a green solid. Yield: 75%. Mp = 152°C. IR (cm^{-1}): 3115, 3071, 2961, 2924, 2850, 1754 (CO), 1680 (CO), 1527, 1272, 1212, 1018, 802, 722. ^{1}H NMR ($CDCl_3$) δ: 7.38 (s, 1 H, H2), 7.27 (dd, J = 8.5, 2.2 Hz, 1 H, H6′), 7.14 (d, J = 2.2 Hz, 1 H, H2′), 6.99 (d, J = 8.5 Hz, 1 H, H5′), 6.75 (d, J = 2.7 Hz, 1 H, H5), 6.60 (d, J = 3.2 Hz, 1 H, H7), 5.95 (m, 1 H, H6), 3.82 (s, 3 H, OMe), 2.28 (s, 3 H, OMe). ^{13}C NMR ($CDCl_3$) δ: 174.1, 168.9, 151.4, 150.7, 139.8, 135.9, 134.1, 128.4, 126.9, 126.4, 124.8, 122.7, 121.1, 115.7, 113.4, 112.7, 55.9, 20.6. Found: C, 63.25; H, 4.26; N, 4.37. $C_{18}H_{13}NO_4S$ requires: C, 63.70; H, 3.86; N, 4.13%.

3-(3-Ethoxycarbonyloxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (**4l**)

To an ice-cooled solution of **4g** (0.2 g, 0.00067 mol) in THF (20 mL) was added portion-wise TEA (0.112 mL, 0.00081 mol), then ethyl chloroformate (0.07 mL, 0.00074 mol). The reaction mixture was then stirred at room temperature overnight, filtered and evaporated to dryness under reduced pressure. The oily residue was taken up in $CHCl_3$ (50 mL) and the solution was washed with an 1 M aqueous HCl solution (100 mL). The organic layer was separated, dried over $CaCl_2$, filtered and the solvent was removed under reduced pressure. The solid was recrystallized from Et_2O/petroleum ether to give pure **4l** as a yellow solid. Yield: 86%. Mp = 138°C. IR (cm^{-1}): 3059, 2961, 2849, 1759 (CO), 1691 (CO), 1530, 1260, 1224, 1015, 801, 740. ^{1}H NMR ($CDCl_3$) δ: 7.37 (s, 1 H, H2), 7.36 (d, J = 8.3 Hz, 1 H, H6′), 7.31 (s, 1 H, H2′), 7.07 (d, J = 8.3 Hz, 1 H, H5′), 6.81 (d, J = 2.4 Hz, 1 H, H5), 6.68 (d, J = 3.2 Hz, 1 H, H7), 6.03 (m, 1 H, H6), 4.34 (q, J = 7 Hz, 2 H, CH_2), 3.92 (s, 3 H, OMe), 1.41 (t, J = 7 Hz, 3 H, Me). ^{13}C NMR ($CDCl_3$) δ: 173.7, 153.1, 151.5, 150.6, 140.1, 135.8, 134.2, 128.2, 127.1, 126.6, 124.8, 122.2, 121.0, 115.7, 113.4, 112.8, 65.2, 56.1, 14.1. Found: C, 61.41; H, 4.02; N, 3.68. $C_{19}H_{15}NO_5S$ requires: C, 61.78; H, 4.09; N, 3.79%.

3-(4-Methoxy-3-pivaloyloxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (**4m**)

To an ice-cooled 5% aqueous solution of sodium hydroxide (10 mL) was added **4g** (0.6 g, 0.002 mol) and then pivalic anhydride (0.53 g, 0.0024 mol). The reaction mixture was heated at 80°C for 2 h, cooled and extracted with Et_2O (2 × 50 mL). The organic layer was separated, washed with water (2 × 100 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (AcOEt/hexane: 1/2) to give pure **4m** as an orange solid. Yield: 77%. Mp = 178°C. IR (cm^{-1}): 3112, 3067, 2980, 2847, 1751 (CO), 1684 (CO), 1470, 1370, 1261, 1129, 806, 724. ^{1}H NMR ($CDCl_3$) δ: 7.46 (s, 1 H, H2), 7.33 (d, J = 8.2 Hz, 1 H, H6′), 7.29 (s, 1 H, H2′), 7.06 (d, J = 8.2 Hz, 1 H, H5′), 6.82 (d, J = 2.3 Hz, 1 H, H5), 6.67 (d, J = 3.3 Hz, 1 H, H7), 5.95 (m, 1 H, H6), 3.92 (s, 3 H, OMe), 1.57 (s, 9 H, 3 Me). ^{13}C NMR ($CDCl_3$) δ: 174.1, 160.7, 151.1, 151.0, 139.5, 135.6, 134.2, 128.6, 127.3, 126.7, 124.9, 122.9, 121.3, 115.9, 113.7, 113.1, 81.2, 55.9, 19.7. Found: C, 66.11; H, 4.75; N, 3.39. $C_{21}H_{19}NO_4S$ requires: C, 66.12; H, 5.02; N, 3.67%.

3[3-(4-Carboxybutylroyloxy)-4-methoxyphenyl]-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (**4n**)

To an ice-cooled solution of **4g** (0.2 g, 0.00067 mol) in pyridine (20 mL), was added glutaric anhydride (0.092 g, 0.00081 mol). The reaction mixture was heated at 80°C for 24 h and evaporated to dryness. The residue was dissolved in AcOEt (50 mL) and the solution was washed twice with a 1 nM aqueous HCl solution (2 × 100 mL), dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure. The residue was recrystallized from MeCN to give pure **4n** as an orange solid. Yield: 76%. Mp = 151°C. IR (cm^{-1}): 1757 (CO), 1725 (CO), 1666 (CO), 1529, 1269, 1126, 1017, 785. ^{1}H NMR ($CDCl_3$) δ: 7.37 (s, 1 H, H2), 7.25 (dd, J = 8.6, 1.9 Hz, 1 H, H6′), 7.11 (d, J = 1.9 Hz, 1 H, H2′), 6.97 (d, J = 8.6 Hz, 1 H, H5′), 6.72 (d, J = 1.9 Hz, 1 H, H5), 6.58 (d, J = 3.4 Hz, 1 H, H7), 5.93 (m, 1 H, H6), 3.81 (s, 3 H, OMe), 2.64 (m, 2 H, CH_2), 2.48 (s, 2 H, CH_2), 2.03 (m, 2 H, CH_2). ^{13}C NMR ($CDCl_3$) δ: 178.4, 174.1, 170.9, 151.3, 150.7, 139.7, 135.8, 134.3, 128.3, 126.9, 126.4, 124.7, 122.6, 121.1, 115.9, 113.4, 112.6, 55.9, 32.8, 32.5, 19.8. Found: C, 61.87; H, 4.46; N, 3.70. $C_{21}H_{17}NO_6S$ requires: C, 61.30; H, 4.16; N, 3.40%.

8*H*-Thieno[3,4-*b*]pyrrolizin-8-one **(5)**

A solution of **16** (0.15 g, 0.00061 mol) in $POCl_3$ (10 mL) was stirred at 70°C for 3 h. After cooling, the reaction mixture was concentrated to give the intermediary iminium salt which was slowly added to a 2.5 nM aqueous NaOH solution (60 mL) and heated at 80°C for 3 h. After cooling, the resulting suspension was extracted with EtOAc (2 × 50 mL), and the combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated to give a dark red solid. This residue was purified by silica gel chromatography, eluting with cyclohexane/EtOAc (2/1) to furnish **5** as a red solid. Yield: 44%. IR (cm^{-1}): 2960,

2924, 2853, 1685 (CO), 1605, 1456, 1262, 1061, 802, 759. ^{1}H NMR ($CDCl_3$) δ: 7.61 (d, J = 2.5 Hz, 1 H, H1), 7.02 (d, J = 2.5 Hz, 1 H, H3), 6.76 (d, J = 3.8 Hz, 1 H, H5), 6.62 (d, J = 2.2 Hz, 1 H, H7), 6.29 (dd, J = 3.8, 2.2 Hz, 1 H, H6). ^{13}C NMR ($CDCl_3$) δ: 173.66, 141.97, 139.37, 138.45, 125.54, 119.64, 115.29, 113.19, 100.76. MS (EI^+) *m/z*: 175.1 (M^+, 100), 147 (93), 82.3 (32).

3-(3-Benzyloxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-ol (**13e**)

To an ice-cooled solution of **4e** (1 g, 0.0026 mol) in anhydrous THF (50 mL), was added $LiAlH_4$ (0.244 g, 0.0064 mol). The reaction mixture was stirred at room temperature for 1 h and then quenched with ice. Alumina was removed by filtration and the filtrate was dried over $MgSO_4$, filtered and evaporated to dryness. The oily residue was dissolved in Et_2O (50 mL) and the solution was washed twice with water (2 × 50 mL). The organic layer was separated, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (AcOEt/cyclohexane: 1/2) to give a solid which was recrystallized from CH_2Cl_2/petroleum ether to give pure **13e** as a beige solid. Yield: 35%. Mp = 118°C. IR (cm^{-1}): 3369 (OH), 1486, 1266, 1248, 1133, 1024, 710. ^{1}H NMR ($CDCl_3$) δ: 7.37 (m, 5 H, H_{Bn}), 7.07 (m, 3 H, H_{Phenyl}), 6.97 (s, 1 H, H2), 6.61 (d, J = 2.0 Hz, 1 H, H5), 6.31 (d, J = 2.0 Hz, 1 H, H7), 6.05 (m, 1 H, H6), 5.67 (d, J = 5.1 Hz, 1 H, H8), 5.18 (s, 2 H, CH_2), 3.46 (s, 3 H, OMe). ^{13}C NMR ($CHCl_3$) δ: 149.7, 148.0, 142.8, 141.9, 136.7, 132.8, 128.7, 128.6, 127.9, 127.2, 126.4, 121.3, 113.9, 113.4, 111.9, 111.0, 106.5, 77.3, 77.0, 76.7, 70.9, 66.9, 56.1. MS (EI^+) *m/z* 389 (M^+ + 1, 64), 339 (65.8), 298 (32), 91 (100).

3-(3-Hydroxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]-pyrrolizin-8-ol (**13g**)

Using the same procedure as for **13e** and starting from a solution of **4g** (0.5 g, 0.0017 mol) in THF (40 mL) and $LiAlH_4$ (0.16 g, 0.0042 mol), **13g** was obtained as a yellow solid after purification by column chromatography (AcOEt/cyclohexane: 1/2). Yield: 45%. Mp = 70°C. IR (cm^{-1}): 3411 (OH), 1509, 1490, 1439, 1277, 1209, 1024, 758, 714. ^{1}H NMR ($CDCl_3$) δ: 7.15 (s, 1 H, H2), 7.11 (d, J = 1.5 Hz, 1 H, H2′), 7.04 (dd, J = 1.5, 9.4 Hz, 1 H, H6′), 6.94 (d, J = 9.4 Hz, 1 H, H5′), 6.83 (d, J = 3.8 Hz, 1 H, H5), 6.35 (d, J = 6.6 Hz, 1 H, H7), 6.13 (m, 1 H, H6), 5.71 (d, J = 5.1 Hz, 1 H, H8), 3.96 (s, 3 H, OMe). ^{13}C NMR ($CHCl_3$) δ: 146.5, 145.8, 142.8, 128.7, 126.4, 120.0, 114.5, 113.6, 110.9, 110.7, 106.6, 77.3, 77.0, 76.7, 67.0, 56.0. MS (EI^+) *m/z* 299 (M^+, 48), 243.1 (22.1), 149 (25), 84.3 (100).

Methyl 4-(pyrrol-1-yl)thiophene-3-carboxylate (**15**)

A solution of 2,5-dimethoxyTHF (0.87 mL, 0.007 mol) in dioxane (30 mL) was stirred for 15 min with 4-chloropyridine hydrochloride (1.05 g, 0.007 mol). Methyl 4-aminothiophene-3-carboxylate **14** (1 g, 0.0064 mol) was added, and the reaction mixture was refluxed for 1.5 h and filtered through a small pad of Celite. The solvent was evaporated to give a brown residue that was dissolved in CH_2Cl_2 (100 mL). The solution was washed with a 1 nM aqueous HCl solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give **15** as a yellow solid that was recrystallized from Et_2O. Yield: 17%. IR (cm^{-1}): 2997, 2951, 2839, 1731 (CO), 1539, 1489, 1454, 1286, 1232, 1083, 727. ^{1}H NMR ($CDCl_3$) δ: 8.13 (d, J = 3.7 Hz, 1 H, H2), 7.21 (d, J = 3.7 Hz, 1 H, H5), 6.86 (m, 2 H, $H_{\alpha pyrrole}$), 6.29 (m, 2 H, $H_{\beta pyrrole}$), 3.77 (s, 3H, OMe). ^{13}C NMR ($CDCl_3$) δ: 162.11, 140.10, 134.16, 128.07, 123.02, 120.41, 109.13, 52.06. MS (EI^+) *m/z*: 207.1 (M^+, 6), 149 (8), 83.9 (100).

3-(Pyrrolidin-1-ylcarbonyl)-4-(pyrrol-1-yl) thiophene (**16**)

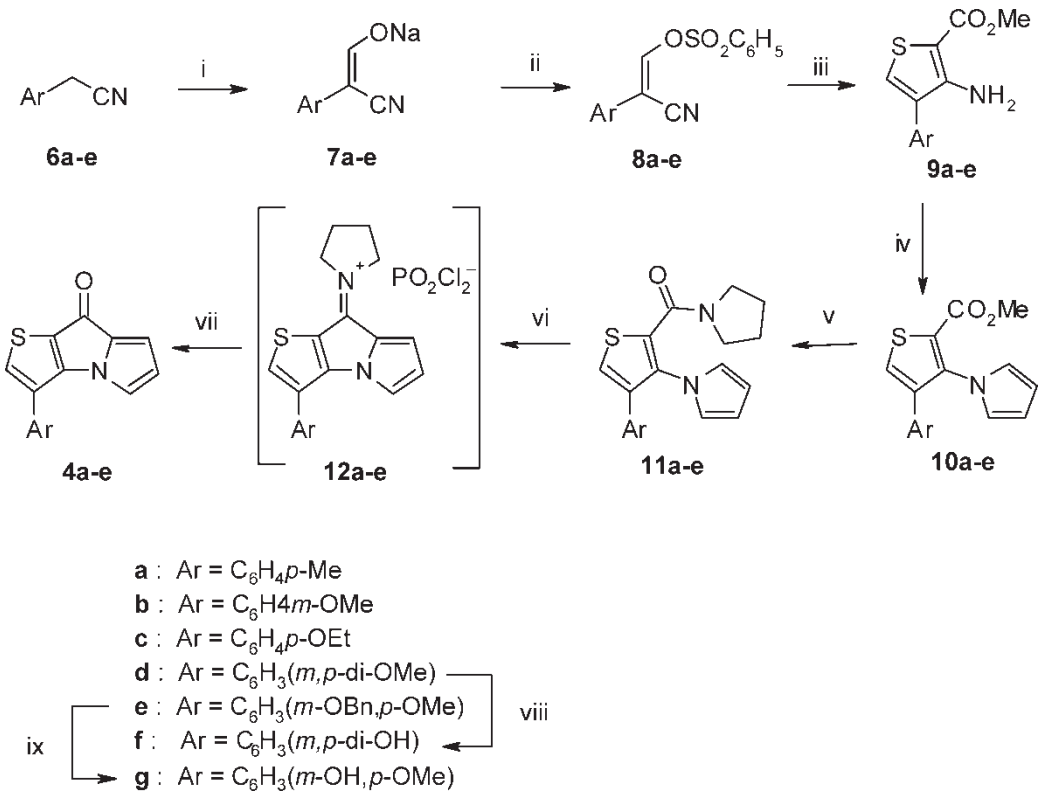
A solution of **15** (0.22 g, 0.0011 mol) in pyrrolidine (15 mL) was refluxed for 12 h. The mixture was cooled and evaporated and the yellow oil was dissolved in $CHCl_3$ (100 mL); finally the solution was washed with an 1 M aqueous HCl solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a black solid. This residue was purified by silica gel chromatography, eluting by cyclohexane/EtOAc (1/2) to furnish **16** as a beige solid. Yield: 69%. IR (cm^{-1}): 3102, 2970, 2869, 1615 (CO), 1557, 1455, 1417, 1223, 1095, 803, 727. ^{1}H NMR ($CDCl_3$) δ: 7.53 (d, J = 3.5 Hz, 1 H, H2), 7.11 (d, J = 3.7 Hz, 1 H, H5), 6.89 (m, 2 H, $H_{\alpha pyrrole}$), 6.25 (m, 2 H, $H_{\beta pyrrole}$), 3.52 (m, 2 H, $H_{\alpha pyrrolidine}$), 2.78 (m, 2 H, $H_{\alpha pyrrolidine}$), 1.79 (m, 2 H, $H_{\beta pyrrolidine}$), 1.65 (m, 2 H, $H_{\beta pyrrolidine}$). ^{13}C NMR ($CDCl_3$) δ: 164.11, 137.41, 133.29, 126.16, 120.73, 114.63, 109.99, 47.23, 45.70, 25.60, 24.19. MS (EI^+) *m/z*: 246.0 (M^+, 86), 176 (24), 149 (100).

Kinase Preparations and Assays

Kinase activities were assayed according to the methodology developed by the Cell Cycle Group of the Station Biologique CNRS, Roscoff, France.[16]

Molecular Modeling

Crystal structures of unphosphorylated GSK-3β in complex with ATP-mimetic inhibitors (staurosporine, indirubine-3′-monoxime, alsterpaullone, I-5) were described recently.[18] As the starting point for our docking studies, the crystal structure of GSK-3β co-crystallized with indirubin-3′-monoxime **2** was used (resolution 2.1 Å).[18] For the docking model, the ligand as well as all the water molecules were removed from the coordinates set. The Gold program [19] was employed to generate an ensemble of docked conformations for reference ligands. This program uses a genetic algorithm to explore conformational spaces and ligand binding modes. The genetic operators were 100 for the population size, 1.1 for



SCHEME 1 Synthesis of compounds **4a**–**g**. Reagents: (i) ethyl formate, MeONa, MeOH; (ii) benzenesulfonyl chloride, DMF; (iii) methyl thioglycolate, MeONa, MeOH; (iv) 2,5-dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (v) pyrrolidine; (vi) $POCl_3$; (vii) 2.5 M NaOH; (viii) BBr_3, CH_2Cl_2; (ix) 33% HBr in AcOH.

the selection pressure (representing the relative probability that the best individual will be chosen as a parent), 5 for the number of subpopulations (island model), 100000 for the maximum number of genetic applications and 2 for the size of the niche used to increase population diversity. The weights were chosen so that crossover and mutation were applied with equal probability (95/95 for the values) and migration was applied 5% of the time. The calculation of the fitness function are described in Gold reference publications (GoldScore and ChemScore).

The compounds **4g** and **13g** were constructed on Sybyl software[†] from a fragment library. The optimisation of the conformation was carried out by molecular mechanics (Tripos force field, Powell method). The termination gradient was fixed to 0.05 kcal/mol/Å (standard deviation of the gradients).

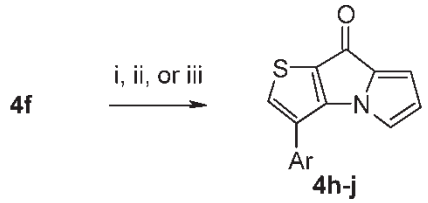
RESULTS AND DISCUSSION

Chemistry

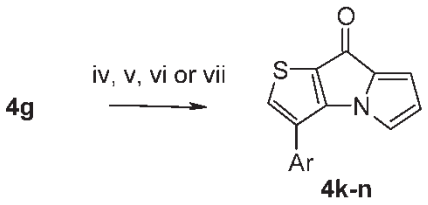
We recently report an efficient chemical sequence leading to some 3-aryl-8*H*-thieno[2,3-*b*]pyrrolizin-8-ones **4a**–**e** in seven steps starting from arylacetonitriles **6a**–**e** (Scheme 1).[20] Treatment of the latter with ethyl formate and sodium methoxide led to the corresponding hydroxyacrylonitrile sodium salts **7a**–**e** which were protected by a benzenesulfonyl group to give **8a**–**e**. Reaction of the latter with methyl thioglycolate and sodium methoxide afforded methyl 3-amino-4-arylthiophene-2-carboxylates **9a**–**e**. Treatment of **9a**–**e** with 2,5-dimethoxytetrahydrofuran and 4-chloropyridine hydrochloride in dioxane gave the pyrrolylthiophene carboxylates **10a**–**e** which led to the corresponding carboxamides **11a**–**e** in refluxing pyrrolidine. Cyclization of the latter was achieved by the action of phosphorus oxychloride to give, after alkaline hydrolysis of the resulting iminium salts **12a**–**e**, the expected thienopyrrolizinones **4a**–**e**. The diphenolic derivative **4f** was further obtained by demethylation of **4d** using boron tribromide, while the hydroxymethoxy compound **4g** resulted furthermore from debenzylation of **4e** under treatment with hydrobromic acid in glacial acetic acid.

The phenolic groups of compounds **4f** and **4g** were acylated by various reagents such as acetic, propionic, pivalic and glutaric anhydrides giving the esters **4h**, **4k**, **4i**, **4m** and **4n** respectively; phosgene and ethyl chloroformate afforded the corresponding carbonates **4j** and **4l** (Scheme 2).

[†]http://www.rcsb.org.

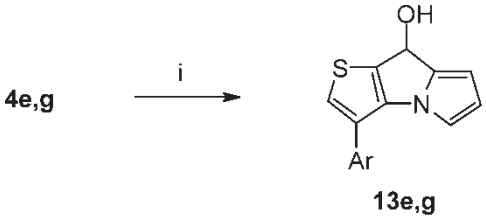


h (i) : Ar = C_6H_3(*m*,*p*-di-OAc)
i (ii) : Ar = C_6H_3(*m*,*p*-di-OCOEt)
j (iii) : Ar = C_6H_3(*m*,*p*-OCO_2)



k (iv) : Ar = C_6H_3(*m*-OAc,*p*-OMe)
l (v) : Ar = C_6H_3(*m*-OCO_2Et,*p*-OMe)
m (vi) : Ar = C_6H_3(*m*-OCOtBu,*p*-OMe)
n (vii) : Ar = C_6H_3(*m*-$OCO(CH_2)_3CO_2H$,*p*-OMe)

SCHEME 2 Synthesis of compounds **4h–n**. Reagents: (i) Ac_2O, THF; (ii) propionic anhydride, THF; (iii) 20% phosgene in toluene, M KOH; (iv) Ac_2O, AcOH; (v) $ClCO_2Et$, TEA, THF; (vi) pivalic anhydride, 1.25 M NaOH; (vii) glutaric anhydride, pyridine.



e : Ar = C_6H_3(*m*-OBn,*p*-OMe)
g : Ar = C_6H_3(*m*-OH,*p*-OMe)

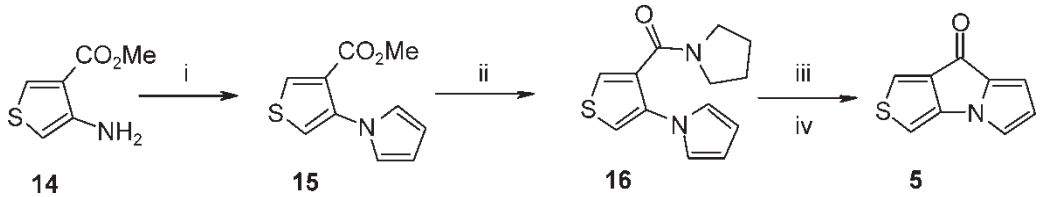
SCHEME 3 Synthesis of compounds **13e–g**. Reagents: (i) $LiAlH_4$, THF.

On the other hand, the pyrrolizinols **13e,g** were obtained from the hydrogenation of the corresponding pyrrolizinones **4e,g** by treatment with lithium aluminium hydride (Scheme 3).

Finally, we undertook the synthesis of the new series 8*H*-thieno[3,4-*b*]pyrrolizin-8-one, taking advantage of the recently commercially available methyl 4-aminothiophene-3-carboxylate **14** (Scheme 4). This useful starting material was involved in the previous sequence leading, via the pyrrolylester **15** and the pyrrolylcarboxamide **16**, to **5**, the regioisomer of the pyrrolizinones **4**.

Biology

Thieno[2,3-*b*]pyrrolizinones **4a–c**, **4e** and **4g–n**, thieno[3,4-*b*]pyrrolizinone **5** and thieno[2,3-*b*]pyrrolizinols **13e,g** were tested against two protein kinases, CDK1/cyclin B and GSK-3. All assays were run in the presence of ATP and the appropriate protein substrates. IC_{50} values were determined from dose-response curves and are provided in Table I. Only 3-(3-hydroxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizinone **4g** exhibited a moderate activity,



SCHEME 4 Synthesis of compound **5**. Reagents: (i) 2,5-dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (ii) pyrrolidine; (iii) $POCl_3$; (iv) 2.5 M NaOH.

TABLE I Inhibitory activities of thienopyrrolizine derivatives against CDK1/cyclin B and GSK-3

	IC_{50} (μM)	
Cpd	CDK1/cyclin B	GSK-3
4a	>50	>50
4b	>50	>50
4c	>50	>50
4e	>50	>50
4g	5.5	1.5
4h	>50	>50
4i	>50	>50
4j	10	17
4k	>50	>50
4l	>50	>50
4m	>50	>50
4n	23	11
5	NT	>50
13e	>50	>50
13g	12	19

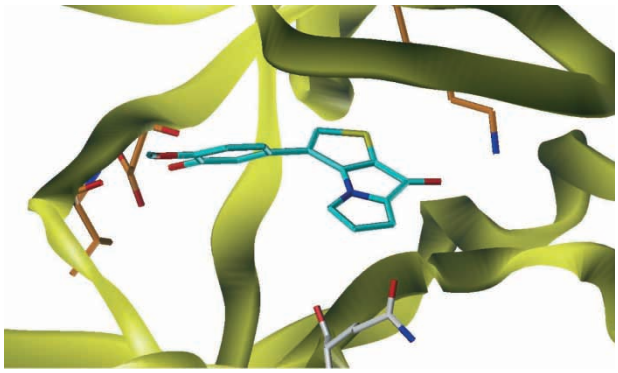


FIGURE 2 Orientation A of **4g** in the active site of GSK-3β (See colour plate at rear).

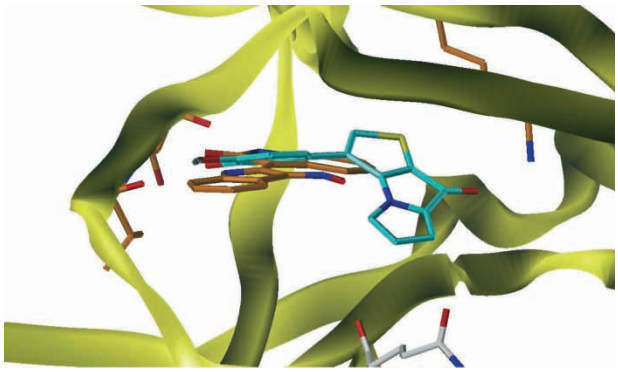


FIGURE 3 Indirubin-3′-monoxime **2** (orange) and **4g** (orientation A-blue) in the active site (See colour plate at rear).

inhibiting CDK1 and GSK-3 in the micromolar range. All the pharmacomodulations concerning either the phenyl substituents or the carbonyl group led to a loss of this activity.

Molecular Modeling

Two orientations of **4g** were obtained after the docking studies.

For the orientation A (Figure 2), the oxygen atoms of the 2-methoxyphenol fragment form two hydrogen bonds with the backbone nitrogen and the carbonyl oxygen of Val135.

These interactions are observed also with indirubin-3′-monoxime **2** (Figure 1, X = NOH, R = H).[18] However, the latter exhibits another hydrogen bond

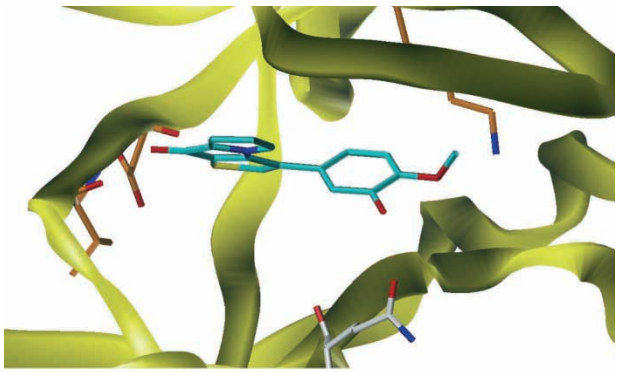
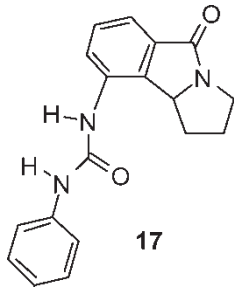


FIGURE 4 Orientation B of **4g** in the active site of GSK-3β (See colour plate at rear).



17

FIGURE 5 Structure of diarylurea **17**.

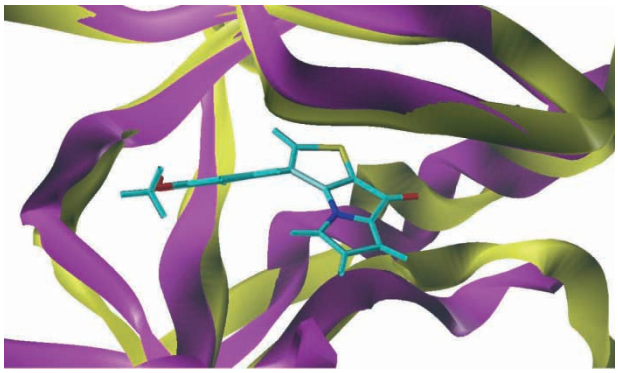


FIGURE 6 Alignment of GSK-3 (yellow) and CDK4 (purple). View of the active site (backbone) (See colour plate at rear).

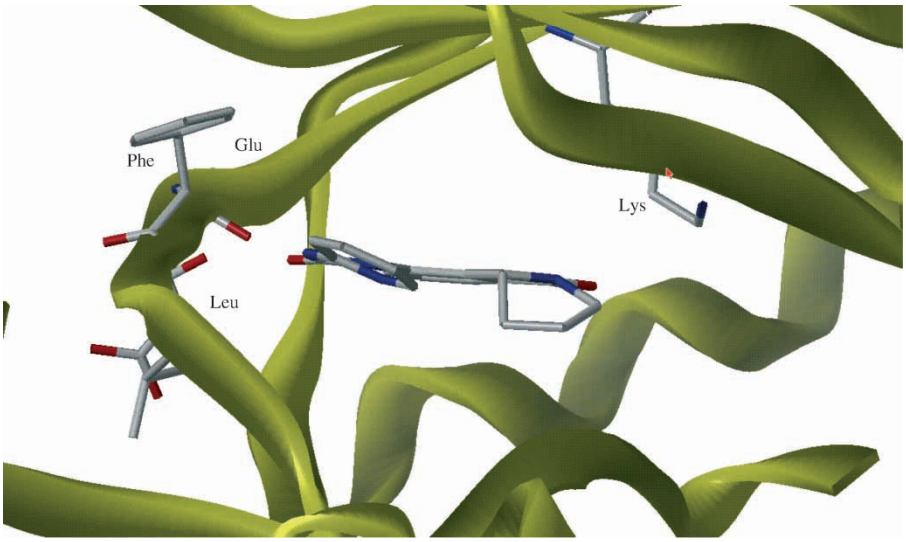


FIGURE 7 Orientation of compound **17** inside the active site of CDK4 (See colour plate at rear).

with the carbonyl oxygen of Asp133. The oxygen of the carbonyl group of **4g** forms an electrostatic interactions with the amino group of Lys85. A close contact between aromatic sulphur and the alkyl chain of Lys85 (hydrophobic interaction) was also observed. The alignment between indirubin-3′-monoxime **2** and **4g** shows that this region is only specific of the latter in term of interactions (Figure 3).

The orientation B (Figure 4) seems to correspond to a rotation of 180° of **4g** compared to the previous one. The oxygen of the carbonyl group forms one hydrogen bond with the backbone nitrogen of Val135 and the oxygen of the *para*-methoxy group engaged in electrostatic interactions with the amino group of Lys85.

The most straightforward method to evaluate the docking accuracy is to inspect how the proposed structure resembles the experimental complex structure. We analysed complexes between kinases and ATP-mimetics inhibitors registered in the Protein Data Bank.[‡] Compound **17**, a CDK4 inhibitor,[21] and **4g** share a similar tricyclic feature (Figure 5).

The three dimensional conformations of the active sites (GSK-3 *vs* CDK4 backbones) are conserved (Figure 6).

Inside the active site, the Lys residue is conserved and the sequence Asp133-Tyr134-Val135 of GSK-3 is equivalent in CDK4 (Glu133-Phe134-Leu135) in term of amino acid types. Compound **17** shows an experimental orientation similar to those of A (Figure 7). This result and the previous observations, *i.e.* three hydrogen bonds for orientation A instead of two (orientation B) and a better position of the sulphur atom in orientation A, are in favour of the latter. This orientation pointed out the importance of the 2-methoxyphenol fragment and by correlation those of the carbonyl group since only **4g** exerted a significant inhibitory activity while its reduced derivative **13g** showed a lower one. Docking studies of the latter led to the same orientation as that for **4g**. However, theoretical calculations of intermolecular interactions (Isostar software[22]) show that the electrostatic interaction between the carbonyl group and cationic amine is stronger than the same interaction between the hydroxy group and cationic amine (-90 kcal/mol *vs* -78 kcal/mol). This last data are in agreement with the biological result and the orientation A.

In conclusion we have identified among our chemical collection a new series some of whose derivatives exhibit moderate CDK1/GSK-3 inhibitory activities. The molecular modeling study, carried out during the course of this work, has particularly pointed out the role played, in the activity of these thienopyrrolizines, by a 2-methoxyphenol moiety. The continuation of this work will consist in replacing the latter by new bioisosteric fragments.

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