Pyrido[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-diones: Synthesis, Cyclometalation, and Protein Kinase Inhibition

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Abstract: Synthetic routes to pyrido[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-diones are disclosed and examples for their subsequent transformations into cyclometalated protein kinase inhibitors are presented.

Key words: pyridocarbazoles, photocyclization, cyclometalation, ruthenium, protein kinase inhibitors

We recently started a research program that aims in exploring the versatility of organometallic and inorganic compounds as structural scaffolds for the design of enzyme inhibitors.1–5 In these molecules, the metal center plays a solely structural role by organizing the organic ligands in the three-dimensional receptor space. We believe that this approach has potentially two important advantages compared to the traditional design of organic inhibitors. First, it gives access to new areas of chemical space that may not be easily accessible with purely organic scaffolds. In this respect, we like to think of a chemically inert metal center as a ‘hypervalent carbon’ with extended structural opportunities. Second, metal complexes are built from a central core and thus may have an advantage in building shape and functional group diversity in an economical fashion.

Our design of metal complexes as protein kinase inhibitors uses the class of indolocarbazole alkaloids [e.g. staurosporine (1)] as a lead structure.4,5 Metal complexes such as 2 bear a pyrido[2,3-a]carbazole bidentate ligand which retains the structural features of the indolocarbazole heterocycle (Figure 1).5 This targets the metal complexes to the ATP-binding site by enabling two H-bonds to the backbone of the hinge between the N-terminal and C-terminal kinase domain, analogous to ATP and conventional organic indolocarbazole inhibitors.6,7 The remaining ligand sphere of the metal gives the opportunity to create interactions with other parts of the ATP-binding site. Potent and specific inhibitors for a particular kinase can now be obtained by assembling elaborate structures around the metal center. For example, the half-sandwich ruthenium complex 3 is a low nanomolar inhibitor for glycogen synthase kinase 3 (GSK-3) (Figure 1).5

In this article, we describe two syntheses of pyrido[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (4) (Scheme 1) and highlight the versatility of our current synthetic strategy by applying it to derivatives in which the indole or pyridine unit is modified. We further demonstrate that these ligands can undergo cyclometalation with ruthenium, leading to molecular scaffolds for the design of highly potent protein kinase inhibitors.

Our initial synthetic strategy to the pyrido[2,3-a]carbazole scaffold 4 is based on the methodology developed by Faul et al. and Andersen et al. (Route A in Scheme 1).8–10 Accordingly, potassium tert-butoxide induced condensation of SEM-protected indole-3-methyl glyoxylate 5 with pyridine-3-acetamide (6) furnishes maleimide 7 in 50% yield. Subsequent oxidative photo-induced cyclization with a medium pressure mercury lamp in a quartz reactor under air and in the presence of catalytic amounts of iodine provides 8 in 63% yield. We observed almost exclusively the illustrated isomer 8, resulting from C–C bond formation between the indole and the ortho-position at the
pyridine. The isomeric product that would have resulted from C–C bond formation between the indole and the para-position at the pyridine was detected only in trace amounts. Finally, SEM-deprotection with LiBF₄ yielded pyridocarbazole in almost quantitative yield. Unfortunately, 4 has a very poor solubility in most organic solvents and is therefore not suitable as a substrate for direct coordination chemistry.

Currently, the TBS-protected pyrido[2,3-a]carbazole 9 (see Scheme 1) is our first choice. It can be synthesized in high yields of 93% starting from 4 by refluxing it with tert-butyldimethylsilyloxyethoxymethene in acetonitrile.

We subsequently developed a shorter and more convenient route which would directly yield the TBS-protected pyrido[2,3-a]carbazole 9. The synthesis is shown in Scheme 1 (Route B). Pyridoindole 10, upon lithiation with LiHMDS, undergoes monosubstitution with TBS-protected dibromomaleimide 11 to afford monobromide 12 in 68% yield. The key step is the following smooth anaerobic photocyclization to pyridocarbazole 9 upon release of HBr (64%). As a side product, a pyridinium salt is formed which results from nucleophilic substitution of the bromide by the pyridine nitrogen. This cationic by-product is easily separable from the desired compound by silica gel chromatography.

This synthetic route is general and can be applied to substituted pyridocarbazole derivatives. For example, the indole substituted derivative 13 was synthesized starting from 2-acetylpyridine 14 (Scheme 2). Hydrazone formation to 15 was followed by Fischer indole synthesis yielding 5-methoxypyridoindole 16 in 63% yield over the two steps. For this Fischer indole synthesis we found trimethylsilyl polyphosphate the Lewis acid of choice. Demethylation to 17 with BBr₃ followed by TBS protection yielded 18. Lithiation of 18, followed by reaction with one equivalent of dibromomaleimide 11 furnished monobromide 19 in 58% yield. Subsequent photolysis under argon provided 13 in a good yield of 78%.
The synthetic route is also applicable to derivative 20 in which the pyridine ring is replaced by an isoquinoline. Starting with 3-acetylisoquinoline (21),16 hydrazone formation to 22 and subsequent Fischer indole synthesis provided the isoquinolinoindole 23 in yields of 40% over the two steps. Compound 23 was lithiated with LiHMDS and reacted with one equivalent of dibromomaleimide to yield the monobromomaleimide substitution product 24 which was directly photocyclized under argon to 20 (84% yield over both steps) (Scheme 3).

The TBS-protected ligands 9, 13, and 20 undergo smooth cyclometalation upon reaction with a suitable metal complex precursor. For example, reaction of 9, 13, and 20 with [Ru(Cp)(CO)(CH₃CN)₂]PF₆ and of 13 with [Ru(Cp*)(CO)(MeCN)₂]PF₆ in the presence of one equivalent of K₂CO₃, followed by TBAF induced TBS-deprotection, yielded the racemic ruthenium complexes 3, and 25–27. Most likely the reaction involves an initial coordination of the pyridyl nitrogen at ruthenium center followed by a nucleophilic attack of the indole nitrogen with subsequent deprotonation. Interestingly, these novel compounds form readily and are very stable. The ruthenium complexes shown in Scheme 4 are completely stable in water, stable in the presence of oxygen, and can withstand millimolar concentrations of thiols.

Ruthenium compounds 3, and 25–27 are potent inhibitors for glycogen synthase kinase 3 (GSK-3). The concentration at which 50% of the enzyme is inhibited (IC₅₀) is 3 nM for 3 as reported recently (measured against the α-isooform of GSK-3).5 Interestingly, increasing the hydrophobic surface area by exchanging the pyridine moiety for an isoquinoline in 25 does not improve the affinity but instead leads to a slight increase of the IC₅₀ to 10 nM. In contrast, the introduction of an hydroxy group at the 5-position of the indole moiety as in 26, decreases the IC₅₀ to 300 pM. Ruthenium compound 26 is one of the most potent compounds available for GSK-3 inhibition.19 In contrast, ruthenium complex 27, which bears the sterically very demanding pentamethylcyclopentadienyl ligand instead of the plain cyclopentadienyl, shows a reduced activity by more than two orders of magnitude, presumably because it cannot fit properly into the ATP pocket of GSK-3 anymore.

In conclusion, two synthetically viable routes for pyrido[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-diones have been discussed. The routes include either an oxidative or nonoxidative photocyclization step. The nonoxidative photocyclization route has proven to be especially useful for the preparation of substituted pyridocarbazoles. These ligands serve as important components for the preparation of cyclometalated protein kinase inhibitors.

NMR spectra were recorded on a Bruker AM-500 (500 MHz), DMX-300 (300 MHz), or DMX-360 (360 MHz) spectrometer. Low-resolution mass spectra were obtained on an LC platform from Micromass using ESI technique. High-resolution mass spectra were obtained with a Micromass AutoSpec instrument using either CI or ES ionization. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer. Solvents and reagents were used as-sup
plied from Aldrich or Acros. Reactions were performed under argon unless otherwise specified.

**Compound 7**

To a stirred solution of 5 (6.82 g, 20.45 mmol) and pyridine-3-acetamide (6; 2.53 g, 18.59 mmol) in DMF (50 mL) at 0 °C, was added dropwise a solution of t-BuOK (6.26 g, 55.77 mmol) in DMF (50 mL). The solution was stirred at 0 °C and was allowed to warm to r.t. The resulting dark red solution was cooled to 0 °C and 20% aq NH4Cl (300 mL) was carefully added. This mixture was extracted with EtOAc (3 ×), and the organic layer was dried (MgSO4) and concentrated. After drying under vacuum to remove the residual DMF, the residue was subjected to silica gel column chromatography with EtOAc–hexane (2:1) as the eluting solvent. The resulting yellow-orange condensation product was isolated in modest yield (3.88 g, 50%).

**IR (film):** 3471, 3266, 3200, 3056, 2922, 2712, 1712, 1624, 1513, 1486, 1396, 1337, 1238, 1178, 1078, 838 cm⁻¹.

**1H NMR (500 MHz, CDCl3):** δ = 7.50 (d, J = 8.1 Hz, 2 H), –0.04 (s, 9 H), 7.28 (ddd, J = 7.7, 1.1, 0.7 Hz, 1 H), 7.24–7.20 (m, 2 H), 1.00 (s, 9 H), 0.50 (s, 9 H).

**13C NMR (125 MHz, CDCl3):** δ = 141.0, 137.0, 136.9, 136.4, 126.3, 123.2, 122.7, 122.6, 121.8, 121.0, 115.5, 111.7, 26.7, 19.4, –3.7.

**HRMS: m/z** calculated for C_{39}H_{50}N_{10}O_{10}S (M + H)^+: 628.3449; found (M + H)^+: 628.3449.

**Compound 8**

A stirred solution of 7 (1.0 g, 2.39 mmol) in MeCN (200 mL) with catalytic amounts of I2 (10 mol%, 0.239 mmol, 60.7 mg) was irradiated with a medium pressure lamp for 2.5 h while air was bubbled through the solution. The resulting suspension was evaporated, and the reaction was repeated three times with this scale. The crude reaction mixtures were combined and purified by silica gel chromatography with CHCl3–MeOH (20:1) to yield 8 (5.2 g, 63%).

**IR (film):** 3334, 2930, 2855, 1704, 1630, 1592, 1529, 1454, 1310, 1250, 1238, 1171, 1078, 838 cm⁻¹.

**1H NMR (500 MHz, DMSO-d6):** δ = 16.96 (s, 1 H), 13.0 (br s, 1 H), 9.44 (dd, J = 8.5, 7.1 Hz, 1 H), 9.44 (s, 3 H), 9.44 (d, J = 8.0 Hz, 1 H), 9.44 (d, J = 4.3, 1.5 Hz, 1 H), 7.65 (dd, J = 8.5, 4.3 Hz, 1 H), 7.58 (m, 2 H), 7.45 (t, J = 8.0 Hz, 1 H), 1.09 (s, 9 H), 0.66 (s, 6 H).

**13C NMR (125 MHz, CDCl3):** δ = 175.6, 174.5, 150.6, 140.1, 139.7, 138.4, 134.7, 130.9, 127.6, 125.8, 123.1, 122.6, 122.1, 122.0, 121.0, 115.5, 111.7, 26.7, 19.4, –3.7.

**Compound 9**

A suspension of pyridoindole 10 (3.36 g, 17.3 mmol) in anhyd THF (45 mL) was stirred at ~−15 °C under argon. Lithium bis(trimethylsilyl)amide (1 M solution in hexanes) (36.6 mL, 36.6 mmol) was added to the solution over 15 min during which time the pale yellow solution became a bright orange suspension. The suspension was allowed to stir for an additional 30 minutes at ~−15 °C. A solution of 11 (6.38 g, 17.3 mmol) in anhyd THF (45 mL) was cooled to 0 °C and then added all at once via syringe to the suspension of lithiated pyridoindole. The suspension was allowed to stir for another 15 min at ~−15 °C and then 45 min at r.t. The dark purple colored slurry was then poured carefully into aq 10% HCl (400 mL) and extracted with EtOAc–hexanes (5:1, later 1:1 or CH2Cl2–MeOH, 20:1) to yield si-

**IR (film):** 2927, 2857, 1752, 1690, 1598, 1528, 1462, 1405, 1339, 1308, 1281, 1230, 1071, 1044 cm⁻¹.

**1H NMR (500 MHz, CDCl3):** δ = 10.36 (br s, 1 H), 9.44 (dd, J = 8.5, 1.4 Hz, 1 H), 9.09 (d, J = 8.0 Hz, 1 H), 9.01 (d, J = 4.3, 1.5 Hz, 1 H), 7.65 (dd, J = 8.5, 4.3 Hz, 1 H), 7.58 (m, 2 H), 7.45 (t, J = 8.0 Hz, 1 H), 1.09 (s, 9 H), 0.66 (s, 6 H).

**13C NMR (125 MHz, CDCl3):** δ = 175.6, 174.5, 150.6, 140.1, 139.7, 138.4, 134.7, 130.9, 127.6, 125.8, 123.1, 122.6, 122.1, 122.0, 121.0, 115.5, 111.7, 26.7, 19.4, –3.7.

**Compound 12**

A suspension of pyridoindole 10 (3.36 g, 17.3 mmol) in anhyd THF (45 mL) was stirred at ~−15 °C under argon. Lithium bis(trimethylsilyl)amide (1 M solution in hexanes) (36.6 mL, 36.6 mmol) was added to the solution over 15 min during which time the pale yellow solution became a bright orange suspension. The suspension was allowed to stir for an additional 30 minutes at ~−15 °C. A solution of 11 (6.38 g, 17.3 mmol) in anhyd THF (45 mL) was cooled to 0 °C and then added all at once via syringe to the suspension of lithiated pyridoindole. The suspension was allowed to stir for another 15 min at ~−15 °C and then 45 min at r.t. The dark purple colored slurry was then poured carefully into aq 10% HCl (400 mL) and extracted with EtOAc (3 ×). The combined organics were washed with sat. aq NaHCO3, brine, and H2O. The organics were dried (MgSO4), filtered, and dried in vacuo. The crude material was subjected to silica gel chromatography with hexanes–EtOAc (first 4:1; then increasing polarity until 100% EtOAc) (sample loading using CH2Cl2). The solvent system was then switched to CH2Cl2–MeOH (10:1) to flush out the remaining orange product 12 which was isolated upon removal of solvent (5.7 g, 68%).

**IR (film):** 3334, 2930, 2855, 1704, 1630, 1592, 1529, 1454, 1352, 1252, 911, 781, 743, 636 cm⁻¹.

**1H NMR (500 MHz, CDCl3):** δ = 16.91, 167.1, 149.7, 149.3, 141.0, 137.0, 136.9, 136.4, 126.3, 122.7, 122.6, 121.8, 120.6, 120.4, 121.2, 104.1, 25.8, 17.8, –3.2.

**HRMS: m/z** calculated for C37H33BrN3O3Si (M + Na)^+: 504.07189; found (M + Na)^+: 504.0713.
**Compound 15**

To a stirred suspension of 4-methoxyphenylhydrazine hydrochloride (5.30 g, 30.4 mmol) in t-BuOH (100 mL) was added 2-acetylpyridine (14; 2.98 mL, 26.4 mmol). The suspension was refluxed for 5 h. After cooling to r.t., the orange precipitate that formed was filtered, washed with cold EtOH and dried under vacuum. The desired product 15 was isolated in quantitative yield (7.33 g) as an orange powder. Characterization data are consistent with a previous report.15

**Compound 16**

To hot trimethylsilyl polyphosphate (65 mL), was added 15 (5.1 g) portionwise over 30 min with stirring. The dark orange solution was stirred for 18 h at 120 °C. The solution was cooled to r.t. and became very thick. The reaction mixture was then neutralized with aq 1 M NaOH. This aqueous solution was extracted with EtOAc (4 ×). The combined organic layers were washed with brine, dried (MgSO4), and concentrated. The crude product was purified by silica gel chromatography (hexanes–EtOAc, 5:1, later 3:1). The desired product 16 was isolated as a pale yellow solid (2.1 g, 71%). Characterization data are consistent with a previous report.15

**Compound 17**

Compound 16 (2.46 g, 11.0 mmol) was dissolved in CH2Cl2 (75 mL). This stirred solution was, added dropwise a 1 M solution of BBr3, in CH2Cl2 (24.13 mL, 24.13 mmol). After the addition was complete, the solution was stirred and allowed to warm to r.t. overnight. After TLC analysis, the reaction was quenched with an aq sat. solution of NaHCO3. The CH2Cl2 layer was removed and washed with fresh NaHCO3 solution. The combined aqueous layers were extracted with EtOAc (3 ×). The combined EtOAc layers were dried (MgSO4), filtered, and evaporated, yielding 17 as a pale yellow solid (2.01 g, 87%). Characterization data are consistent with a previous report.15

**Compound 18**

IR (film): 3265, 2936, 2854, 1593, 1545, 1440, 1217 cm–1.

**Compound 19**

A solution of 18 (1.8 g, 5.6 mmol) in THF (16 mL) was purged with argon, and cooled to –15 °C. With stirring, a 1 M solution of lithium bis(trimethylsilyl)amide in THF (11.2 mL, 11.2 mmol) was added dropwise over 15 min. During the course of the addition, the color of the solution changed from pale yellow to deep purple. After the addition was complete, the mixture was stirred at –15 °C for 45 min. To this mixture, was added quickly a cold solution of 11 (2.05 g, 5.6 mmol) in THF (16 mL), after which the mixture was stirred for 20 min at –15 °C and 45 min at r.t. The reaction was quenched with 1 M HCl and extracted with EtOAc (3 ×). The combined organic layers were washed with an aq sat. solution of NaHCO3, distilled H2O, and brine, dried (MgSO4), and concentrated. The crude material was further purified by silica gel chromatography with hexanes–EtOAc (10:1, later 5:1) yielding 19 as a bright orange solid (1.96 g, 58%).

**Compound 20**

A stirred solution of 19 (334 mg, 0.55 mmol) in MeCN (200 mL) was irradiated with a medium pressure mercury lamp through a pyrex filter for 3 h while argon was bubbled through the solution. The resulting suspension was concentrated and subjected to silica gel chromatography. Some impurity was eluted with 100% CH2Cl2, and the desired product with CH2Cl2–MeOH (100:1, later 50:1). Compound 20 was isolated as a bright yellow solid (229 mg, 78%).

**Compound 21**

To a suspension of 19 (350 mg, 0.55 mmol) in CH2Cl2 (100 mL) were added 2-acetylpyridine (7.9 mL, 45.24 mmol) at 0 °C under vigorous stirring. The mixture was then neutralized with aq 1 M NaOH. This aqueous solution was extracted with EtOAc (3 ×). The combined EtOAc layers were dried (Na2SO4), filtered, and evaporated, yielding 20 as a pale yellow solid (2.05 g, 55%).

**Compound 22**

A stirred suspension of 21 (3.39 g, 19.82 mmol) in absolute EtOH (4.8 mL) was added phenyl hydrazine (4.8 mL, 19.82 mmol). The suspension was refluxed for 2 h during which time the color of the reaction mixture became yellow. Upon cooling, a yellow precipitate was formed. The mixture was cooled to 0 °C for 30 min, and then the yellow precipitate was collected via vacuum filtration. The solid was washed with cold EtOH, then dried under vacuum to provide 22 as a yellow solid (4.4 g, 85%).
IR (film): 3438, 3274, 2926, 2849, 1949, 1747, 1698, 1690, 1575, 1566, 1465, 1438, 1377, 1340, 1304, 1269, 1269, 1205, 1176, 1165, 1158, 1142, 82.6

Compound 26
A suspension of ligand 13 (51 mg, 0.096 mmol), K₂CO₃ (13.3 mg, 0.096 mmol) and [Ru(Cp)(CO)(CH₃CN)]PF₆ (40.5 mg, 0.096 mmol) was heated in MeCN (5 mL) to 55 °C overnight. The resulting red/purple solution was then dried in vacuo, redissolved in CH₂Cl₂ (5 mL) and subsequently desilylated with the addition of 1 M solution of TBAF in THF (248 µL, 0.992 mmol) for 15 min at r.t. Glacial AcOH (2.9 mL, 0.050 mmol) was added and the solution stirred at r.t. for 10 min. The solution was then dried in vacuo and purified by silica gel chromatography using EtOAc/hexanes (1:1) as eluant to afford 26 as a purple solid (31 mg, 67%).

IR (film): 3438, 3274, 2926, 2849, 1949, 1747, 1698, 1690, 1575, 1566, 1465, 1438, 1377, 1340, 1304, 1269, 1269, 1205, 1176, 1165, 1158, 1142, 82.6
\( ^{13}C \) NMR (extracted from hmbc and hmqc two-dimensional experiments) (600 MHz/150 MHz, DMSO-\( d_6 \)): \( \delta = 170.0, 155.0, 150.9, 146.3, 143.4, 132.1, 130.5, 123.2, 122.1, 120.5, 115.7, 113.7, 110.2, 107.5, 80.9 \).

**Compound 27**

A suspension of ligand 13 (27 mg, 0.052 mmol), K\(_2\)CO\(_3\) (7 mg, 0.052 mmol) and [RuCl\(_2\)(CO)(CH\(_3\)CN)\(_2\)]\(\text{PF}_6\) (25 mg, 0.052 mmol) was heated in MeCN (3 mL) to 55 °C overnight. The resulting red/purple solution was then dried in vacuo, redissolved in CH\(_2\)Cl\(_2\)–MeOH (50:1) as eluent to afford (8.5 mg, 54%).

The reaction was then quenched by the addition of glacial AcOH (1 H), 7.10 (dd, J = 8.3, 1.0 Hz, 1 H), 8.91 (dd, J = 8.8, 2.5 Hz, 1 H), 1.70 (s, 15 H).

IR (film): 3310, 2920, 1742, 1694, 1591, 1532, 1499, 1472, 1423, 1342, 1213, 1010 cm\(^{-1}\).

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**References**


