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Synthesis and Anti-Tuberculosis Activity of Olivacine and Oxygenated Derivatives

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Abstract: The tetracyclic pyrido[4,3-b]carbazole olivacine and four of its oxygenated derivatives have been synthesized by a late-stage palladium-catalyzed Heck-type cyclization of the pyrrole ring as key step. In a test for inhibition of the growth of Mycobacterium tuberculosis 9-methoxyolivacine showed the most significant anti-TB activity with an MIC90 value of 1.5 μM.

Keywords: anti-TB activity; catalysis; cyclization; olivacine; palladium; pyrido[4,3-b]carbazoles

1. Introduction

The pyrido[4,3-b]carbazole alkaloid olivacine (1, Figure 1) was first isolated in 1958 by Schmutz et al. [1] and its structural assignment was confirmed by total synthesis only two years later [2]. The tetracyclic alkaloid 1 and many structurally related compounds, for example the isomeric natural product ellipticine (2), show useful biological activities such as antitumor activity based on DNA intercalation, topoisomerase II inhibition and antimalarial activity [3–7]. Since the 1980s, A-ring oxygenated derivatives of ellipticine (2) have attracted much attention because of their anti-tumor activity [8]. Despite its side effects, elliptinium acetate (3) has reached the status of a licensed drug for the treatment of advanced breast cancer [9]. Diverse total syntheses of olivacine (1) have been reported [10–15]. Surprisingly, the pharmacological potential of olivacine (1) and its oxygenated derivatives (for example 4 and 5) has been much less investigated [16].

![Figure 1. Pyrido[4,3-b]carbazole alkaloids and oxygenated derivatives.](image-url)

Although 9-hydroxyolivacine (5) is the main metabolite of olivacine (1) [3], only few derivatives of olivacine (1) with substituents exclusively at the A-ring have been described in the literature [3,11]. This may be due to the fact that the syntheses of pyrido[4,3-b]carbazoles usually involve the
annulation of an isoquinoline or a pyridine at an indole or carbazole framework [8,10,11]. Thus, a facile variation of the substitution pattern at ring A is not easy to accomplish. Herein, we present a novel route for the synthesis of the tetracyclic pyrido[4,3-b]carbazole framework [17].

2. Results and Discussion

For a convergent access to various A-ring substituted derivatives, we envisaged a late-stage B-ring construction of the pyrido[4,3-b]carbazole framework. Therefore, we thought to apply the two-step sequence of palladium-catalyzed reactions developed by our group for carbazole assembly: synthesis of a diarylamine via Buchwald–Hartwig coupling of appropriate anilines 7 with a substituted isoquinoline 8 followed by oxidative cyclization to the pyrido[4,3-b]carbazoles 6 (Scheme 1) [11]. The isoquinoline 8 would be available by Bischler–Napieralski cyclization of the arylethylamine 9 via the corresponding acetamide. Henry reaction of an appropriately substituted benzaldehyde 10 and subsequent reduction should afford the arylethylamine 9. As the Bischler–Napieralski reaction works best on electron rich aromatic systems, we decided to start from the commercially available methoxy-substituted benzaldehyde 11 (Scheme 2) and to transform the methoxy group into a suitable leaving group at a later stage of our synthesis.


2.1. Total synthesis

Starting from commercial benzaldehyde 11, which can also be obtained almost quantitatively in one step from the much cheaper m-anisaldehyde [18], amide 12 is prepared by a three-step sequence of Henry reaction, LAH reduction and N-acetylation (Scheme 2) [19]. Bischler–Napieralski cyclization using phosphorus oxychloride led to the corresponding dihydroisoquinoline which was fully aromatized to 6-methoxy-1,5-dimethylisoquinoline (13) by dehydrogenation with palladium on charcoal in the presence of cyclohexene as additive. Cleavage of the methyl ether afforded the isoquinolinol which on reaction with trifluoromethanesulfonic anhydride provided the known isoquinolinyl triflate 14 [20] in 58% yield over seven steps.

Scheme 2. Synthesis of the triflate 14. Reagents and conditions: (a) MeNO₂, NH₄OAc, AcOH, 80 °C, 110 min, 77%; (b) LiAlH₄, THF, 0 °C to reflux, 19.5 h, 92%; (c) Ac₂O, DMAP, pyridine, 0 °C, 4 h, 99%; (d) POCl₃, reflux, 1 h, 99%; (e) Pd/C (10%), cyclohexene, PhMe, reflux, 1.5 h, 100%; (f) pyridinium chloride, microwave (300 W), 155 °C, 30 min, 96%; (g) Tf₂O, pyridine, MeCN, 0 °C, 20 h, 87%.

Buchwald–Hartwig coupling of the triflate 14 and aniline (15) provided the diarylamine 16 (Scheme 3). However, the oxidative cyclization to the pyrido[4,3-b]carbazole framework proved to be very difficult [21]. Diverse attempts to optimize this reaction failed: using different reaction temperatures, different solvents (HOAc, HOPiv, dioxane, toluene), catalytic amounts of palladium(II) acetate in the presence of different re-oxidants, or stoichiometric amounts of palladium(II) acetate [22–24]. These experiments resulted to a large extent in decomposition and led to olivacine (1) in only low to moderate yields with poor reproducibility.
Scheme 3. Synthesis of olivacine (1) via oxidative cyclization. Reagents and conditions: (a) cat. Pd(OAc)$_2$, cat. XPhos, Cs$_2$CO$_3$, PhMe, reflux, 48 h, 100%; (b) 1.1 equiv. Pd(OAc)$_2$, AcOH, 80–100 °C, 24 h, argon, 9–49%.

Therefore, we decided to apply a Heck-type cyclization for the formation of the crucial carbon–carbon bond of the central pyrrole ring. This approach was already described by Sakamoto and coworkers in 1999 [25]. Buchwald–Hartwig coupling of the triflate 14 with the commercially available o-chloroanilines 17a–c led to the corresponding diarylamines 18a–c in 83–94% yield (Scheme 4). Compound 18a was structurally confirmed by an X-ray analysis (Figure 2).

Scheme 4. Synthesis of the pyrido[4,3-b]carbazoles 1, 4, and 5. Reagents and conditions: (a) cat. Pd(OAc)$_2$, cat. XPhos, Cs$_2$CO$_3$, PhMe, reflux, 1–5 h, 83–94% (18a–c); (b) cat. Pd(OAc)$_2$, P(tBu)$_3$·HBF$_4$, K$_2$CO$_3$, DMF, 140 °C, 20–35 min, 62–71% (1, 19b, 19c), 3–12% (20a–c); (c) HBr(aq), reflux, 24 h, 70–84% (4, 5).

Figure 2. Molecular structure of the diarylamine 18a in the crystal (ORTEP plot showing thermal ellipsoids at the 50% probability level).
The cyclization reaction of the diarylamine 18a with catalytic amounts of palladium(II) acetate in the presence of P(tBu)3·HBF4 and K2CO3 in DMA at 110 °C and in DMF at 120 °C proceeded very slowly [26,27]. Hydrodehalogenation leading to compound 16 was the major side reaction. Using only slightly higher temperatures (130–140 °C), the reaction proceeded much faster (Table 1). Finally, using larger amounts of the catalyst combined with shorter reaction times, olivacine (1) was obtained in 71% yield. The structure was confirmed by an X-ray crystal structure determination (Figure 3).

Table 1. Optimization of the Heck-type cyclization of 18a.

<table>
<thead>
<tr>
<th>Pd(OAc)2 (equiv.)</th>
<th>ligand1 (equiv.)</th>
<th>K2CO3 (equiv.)</th>
<th>solvent</th>
<th>temp. (°C)</th>
<th>time (h)</th>
<th>yield (%)</th>
<th>RSM2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.2</td>
<td>DMA</td>
<td>110</td>
<td>24</td>
<td>11</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>0.2</td>
<td>DMA</td>
<td>130</td>
<td>1.5</td>
<td>46</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.4</td>
<td>DMA</td>
<td>120</td>
<td>3.0</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>1.0</td>
<td>DMA</td>
<td>120</td>
<td>45</td>
<td>46</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.4</td>
<td>DMF</td>
<td>140</td>
<td>3.0</td>
<td>62</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>0.3</td>
<td>0.6</td>
<td>DMF</td>
<td>140</td>
<td>0.5</td>
<td>71</td>
<td>–</td>
</tr>
</tbody>
</table>

1 P(tBu)3·HBF4 was used as ligand; 2 RSM = reisolated starting material; 3 reagents added in portions of 0.1 equiv. Pd(OAc)2, 0.2 equiv. ligand, 2 equiv. K2CO3 after 0, 1, 3, 6, 30 h of reaction time.

Figure 3. Molecular structure of olivacine (1) in the crystal (ORTEP plot showing thermal ellipsoids at the 50% probability level).

Application of these conditions to the cyclization of the diarylamines 18b and 18c provided 8-methoxyolivacine (19b) and 9-methoxyolivacine (19c) in 65% and 62% yield, respectively (Scheme 4). The structure for 8-methoxyolivacine (19b) was additionally confirmed by an X-ray analysis of single crystals (Figure 4). 9-Methoxyolivacine (19c) is a natural product which was isolated in 1967 from the bark of the coastal Venezuelan tree Aspidosperma vargasii A. DC. [28]. Interestingly, the 11bH-pyrido[3,4-c]carbazoles 20a–c containing a quaternary carbon atom were obtained as by-products of the cyclization reactions of the diarylamines 18a–c in up to 12% yield. The structural assignments for the 11bH-pyrido[3,4-c]carbazoles 20a–c were supported by 2D NMR (COSY, HMBC, HSQC, NOESY) spectroscopic studies (see Supplementary Materials). The compounds 20a–c result from an attack at the C5 carbon atom of the isoquinoline moiety. Cleavage of the methyl ether of 19b and 19c provided 8-hydroxyolivacine (4) and 9-hydroxyolivacine (5) in 84% and 70% yield, respectively. For biological testing, the products were additionally purified by HPLC.
2.2. Biological activity

A weak anti-tuberculosis (anti-TB) activity was described in early reports for some simple tricyclic carbazole alkaloids [29–31]. Based on that work, we investigated the anti-TB activity of a range of oxygenated carbazole alkaloids and their derivatives and found very promising results for several compounds [32–34]. Therefore, we also tested olivacine (1) and its oxygenated derivatives 4, 5, 19b and 19c for their anti-TB activities (Table 2). In a preliminary activity test against Mycobacterium tuberculosis only two of the five pyrido[4,3-b]carbazoles, namely olivacine (1) and 9-methoxyolivacine (19b), showed significant effects and have been studied further. The minimum concentrations effecting a 90% inhibition of growth (MIC90) of M. tuberculosis strain H37Rv were determined by the microplate alamar blue assay (MABA) [35,36]. The in vitro cytotoxicity towards mammalian (vero) cells was determined as described previously [35,37].

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC90 [µM]</th>
<th>IC50 [µM]</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olivacine (1)</td>
<td>4.7</td>
<td>18.05</td>
<td>3.8</td>
</tr>
<tr>
<td>8-Hydroxyolivacine (4)</td>
<td>n.d.¹</td>
<td>n.d.</td>
<td>–</td>
</tr>
<tr>
<td>9-Hydroxyolivacine (5)</td>
<td>n.d.¹</td>
<td>n.d.</td>
<td>–</td>
</tr>
<tr>
<td>8-Methoxyolivacine (19b)</td>
<td>n.d.¹</td>
<td>n.d.</td>
<td>–</td>
</tr>
<tr>
<td>9-Methoxyolivacine (19c)</td>
<td>1.5</td>
<td>24.5</td>
<td>16.3</td>
</tr>
<tr>
<td>3-Methoxy-2-methylcarbazole-1,4-quinone⁶</td>
<td>4.0</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>Isoniazid⁵</td>
<td>0.24</td>
<td>&gt;50</td>
<td>&gt;208</td>
</tr>
<tr>
<td>Rifampicin⁵</td>
<td>0.02</td>
<td>&gt;50</td>
<td>&gt;2500</td>
</tr>
</tbody>
</table>

¹ Minimum inhibitory concentrations [µM] against M. tuberculosis H37Rv in the MABA assay; values are the mean of three replicate experiments; n.d. = not determined. ² Cytotoxicity corresponding to the concentration [µM] effecting 50% decrease in tetrazolium dye reduction by vero cells (African green monkey kidney cells); values are the mean of three replicate experiments; for experiments giving a value higher than the max. conc. used, >50 µM is denoted. ³ Selectivity index: SI = IC50/MIC90. ⁴ These compounds showed no significant inhibition in a preliminary assay. ⁵ 3-Methoxy-2-methylcarbazole-1,4-quinone, isoniazid and rifampicin (rifampin) were used as positive control; solvent was used as negative control.
The MIC\textsubscript{90} value for 3-methoxy-2-methylcarbazole-1,4-quinone served as benchmark for comparison with the anti-TB activities of carbazoles found in our previous studies [34]. Although olivacine (1) shows an activity comparable to our benchmark compound, the SI value is considerably lower (SI = 3.8) due to its toxicity. However, 9-methoxyolivacine (19a) exhibits a strong anti-TB activity (MIC\textsubscript{90} = 1.5 \mu M) combined with a lower cytotoxicity towards mammalian cells which leads to a very good selectivity index (SI = 16.3).

3. Materials and Methods

3.1. General

All reactions were carried out in oven-dried glassware using anhydrous solvents under an argon atmosphere, unless stated otherwise. CH\textsubscript{2}Cl\textsubscript{2}, THF, and toluene were dried using a solvent purification system (MBr aun-SPS). Petroleum ether used refers to the hydrocarbon mixture with a boiling range of 40–65 °C. Pd(OAc)\textsubscript{2} was recrystallized from glacial AcOH. All other chemicals were used as received from commercial sources. A CEM Discover microwave reactor was utilized for reactions taking place under microwave irradiation. Flash chromatography was performed using silica gel from Acros Organics (0.035–0.070 mm). Alox N was obtained from Merck KGaA. TLC was performed with TLC plates from Merck (60 F254) using UV light for visualisation. Melting points were measured on a Gallenkamp MPD 350 melting point apparatus. Ultraviolet spectra were recorded on a PerkinElmer 25 UV/Vis spectrometer. Fluorescence spectra were obtained using a Varian Cary Eclipse spectrometer. IR spectra were recorded on a Thermo Nicolet Avatar 360 FT-IR spectrometer using the ATR method (Attenuated Total Reflectance). NMR spectra were measured on a Bruker DRX 500 and Avance III 600 spectrometers. Chemical shifts \( \delta \) are reported in parts per million (ppm) with the solvent signal as internal standard. Standard abbreviations were used to denote the multiplicities of the signals. MS and HRMS (EI) were recorded on a Finnigan MAT-95 spectrometer (electron impact, 70 eV) or by GC/MS-coupling using an Agilent Technologies 6890 N GC System equipped with a 5973 Mass Selective Detector (electron impact, 70 eV). ESI-MS spectra were recorded on an Esquire LC with an ion trap detector from Bruker. Positive and negative ions were detected. ESI-HRMS were recorded using a Q-TOF 6538 (Agilent). Elemental analyses were measured on an EuroVector EuroEA3000 elemental analyser. X-ray crystal structure analyses were performed with a Bruker-Nonius Kappa CCD that was equipped with a 700 series Cryostream low temperature device from Oxford Cryosystems. SHELXS-97 [38], SADABS version 2.10 [39], SHELXL-97 [40], POV-Ray for Windows version 3.7.0.msvc10.win64, and ORTEP-3 for Windows [41] were used as software.

3.2. Procedures

1-Methoxy-2-methyl-3-(2-nitrovinyl)benzene. Nitromethane (427 mg, 6.99 mmol) and freshly sublimated ammonium acetate (433 mg, 5.62 mmol) were added to a solution of 3-methoxy-2-methylbenzaldehyde (11, 800 mg, 5.33 mmol) in acetic acid (645 mg, 10.74 mmol) and the mixture was stirred at 80 °C for 1 h 50 min. After cooling to room temperature, the precipitate was dissolved by adding ethyl acetate. The mixture was transferred to a separatory funnel, washed twice with water and brine. The aqueous layer was extracted with ethyl acetate, the combined organic layers were dried (magnesium sulfate) and the solvent was evaporated. Purification of the residue by column chromatography (silica gel, petrol ether, ethyl acetate, 1% to 15% ethyl acetate) provided 1-methoxy-2-methyl-3-(2-nitrovinyl)benzene (791 mg, 4.09 mmol, 77%) as yellow crystals. m.p. 97–98 °C; UV (MeOH): \( \lambda = 205, 228, 251, 317 \text{ nm} \); IR (ATR): \( \nu = 3116, 2959, 2920, 2838, 1901, 1820, 1697, 1653, 1627, 1594, 1573, 1541, 1498, 1477, 1450, 1331, 1260, 1244, 1102, 1080, 1007, 968, 893, 873, 844, 806, 777, 725, 693 \text{ cm}^{-1} \); \( ^{1} \text{H NMR} (500 \text{ MHz}, \text{CDCl}_{3}) \): \( \delta = 2.33 (s, 3 \text{H}), 3.86 (s, 3 \text{H}), 6.96 (d, \ J = 8.2 \text{ Hz}, 1 \text{H}), 7.10 (d, \ J = 7.8 \text{ Hz}, 1 \text{H}), 7.22 (t, \ J = 8.0 \text{ Hz}, 1 \text{H}), 7.48 (d, \ J = 13.5 \text{ Hz}, 1 \text{H}), 8.33 (d, \ J = 13.5 \text{ Hz}, 1 \text{H}); \( ^{13} \text{C NMR} (125 \text{ MHz}, \text{CDCl}_{3}) \): \( \delta = 11.96 (\text{CH}_{3}), 55.84 (\text{CH}_{3}), 113.12 (\text{CH}), 119.25 (\text{CH}), 127.11 (\text{CH}), 128.40 (\text{C}), 130.13 (\text{C}), 137.25 (\text{CH}), 138.20 (\text{CH}), 158.29 (\text{C}); \) MS (EI): \( m/z (\%) = 193 (100, [\text{M}]^{+}), 178 (6), 161 (7), 146 (70), 131 (52), 115 (54), 103 (67), 91 (37), 77 (47), 63 (18), 51 (18); \) HRMS: calcd for...
2-(3-Methoxy-2-methylphenyl)ethanamine. Over a period of 1 h a solution of 1-methoxy-2-methyl-3-(2-nitrovinyl)benzene (6.79 g, 35.2 mmol) in THF (95 mL) was added to a suspension of lithium aluminum hydride (6.83 g, 180 mmol) in THF (360 mL) at 0 °C. The cooling bath was removed and the mixture was heated for 30 min at room temperature and 18 h at reflux. A second portion of lithium aluminum hydride (0.35 g, 9.1 mmol) was added to the slightly reddish colored solution and the mixture was heated at reflux for an additional hour. After cooling to room temperature, the reaction mixture was carefully quenched with saturated aqueous ammonium chloride and the pH value was adjusted to 9. Diethyl ether was added and the mixture was transferred into a separatory funnel. Still under argon, the layers were separated and the aqueous layer was extracted three times with diethyl ether. The combined organic layers were washed with water and brine, dried (magnesium sulfate) and the solvent was evaporated to provide 2-(3-methoxy-2-methylphenyl)ethanamine (5.33 g, 32.3 mmol, 92%) as a yellow oil. UV (MeOH): λ = 204, 218, 273, 280 nm; IR (ATR): v = 3402, 2989, 2923, 2848, 2659, 2480, 2065, 1658, 1604, 1581, 1510, 1463, 1395, 1293, 1256, 1194, 1171, 1149, 1122, 1096, 1006, 953, 875, 789, 776, 763, 719 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ = 2.22 (s, 3H), 2.30–3.19 (m, 4H), 3.82 (s, 3H), 6.77 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 7.6 Hz, 1H); 13C NMR (150 MHz, methanol-D₄): δ = 11.47 (CH₃), 23.51 (CH₃), 32.62 (CH₂), 40.35 (CH₂), 55.49 (CH₃), 109.10 (CH), 121.76 (CH), 125.07 (C), 126.59 (CH), 135.84 (C), 158.03 (C); MS (ESI, +10 V) m/z = 149.0 [M–NH₃]+, 166.0 [M+H]+, 331.2 [2M+H]+; HRMS: calcd for C₁₀H₁₅NO: 165.1153, found: 165.1144.

2-(3-Methoxy-2-methylphenyl)ethylacetamide (12). DMAP (14 mg, 0.11 mmol) was added to a solution of 2-(3-methoxy-2-methylphenyl)ethanamine (230 mg, 1.14 mmol) in pyridine (4.5 mL) and the mixture was cooled to 0 °C. Acetic anhydride (140 µL, 1.5 mmol) was added dropwise over a period of five minutes and the reaction mixture was stirred for four hours. The solvent was evaporated and the raw material was purified by chromatography (Alox N, 5% H₂O; ethyl acetate) to provide 2-(3-methoxy-2-methylphenyl)ethylacetamide (123 mg, 0.75 mmol, 99%) as a light yellow solid. M.p. 84–85 °C; UV (MeOH): λ = 205, 219, 229, 271, 279 nm; IR (ATR): v = 3267, 3085, 2932, 2836, 2030, 2009, 1976, 1716, 1659, 1630, 1564, 1508, 1489, 1472, 1459, 1345, 1396, 1370, 1298, 1285, 1247, 1201, 1180, 1110, 1092, 1037, 1013, 896, 812, 776, 748, 723, 701, 651, 606 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 1.95 (s, 3H), 2.19 (s, 3H), 2.64 (t, J = 6.9 Hz, 2H), 3.46 (q, J = 6.9 Hz, 2H), 3.82 (s, 3H), 5.46 (br s, 1H), 6.76 (d, J = 7.9 Hz, 2H); ¹³C NMR (125 MHz, CDCl₃): δ = 11.35 (CH₃), 23.51 (CH₃), 33.38 (CH₃), 39.87 (CH₂), 55.64 (CH₃), 108.61 (CH), 121.89 (CH), 125.66 (C), 126.63 (CH), 138.38 (C), 158.09 (C), 170.21 (C=O); MS (ESI, +10 V) m/z = 208.0 [M+H]+, 415.1 [2M+H]+, 437.1 [2M+Na]+; HRMS: calcd for C₁₀H₁₅NO: 165.1259, found: 165.1248; elemental analysis: calcd for C₁₀H₁₅NO: C: 69.54, H: 9.72, N: 7.67; found C: 69.04, H: 8.73, N: 6.78.

6-Methoxy-1,5-dimethyl-3,4-dihydroisoquinoline. Phosphorus oxychloride (1.9 mL, 21 mmol) was added to a refluxing solution of acetamide 12 (433 mg, 2.09 mmol) in freshly distilled chloroform (23 mL) and the mixture was stirred for one hour. Subsequently, solvent and excess phosphorus oxychloride were removed under vigorous stirring by a nitrogen stream through a pair of soda lye filled gas washing bottles. The remaining oily raw product was dissolved in ethyl acetate. Soda lye (10%) was added and the pH value was adjusted to 8–9 using saturated aqueous ammonium chloride. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried (magnesium sulfate) and the solvent was evaporated. Purification of the crude product by chromatography (Alox N, 5% H₂O; ethyl acetate + 3% triethylamine) afforded 6-methoxy-1,5-dimethyl-3,4-dihydroisoquinoline (393 mg, 2.08 mmol, 99%) as a yellow solid. M.p. 57–58 °C (subl.); UV (MeOH): λ = 229, 274, 319 nm; IR (ATR): v = 3002, 2939, 2838, 1735, 1699, 1629, 1594, 1576, 1539, 1507, 1482, 1435, 1368, 1291, 1258, 1184, 1149, 1101, 1015, 922, 901, 873, 805, 751, 700, 666, 637 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 2.15 (s, 3H), 2.35 (t, J = 1.4 Hz, 3H), 2.64 (t, J = 7.4 Hz, 2H); ³J (tq, J = 7.4, 1.4 Hz, 2H), 3.85 (s, 3H), 6.75 (d, J = 8.5 Hz,
combined organic layers were washed with water and brine, and then dried (sodium sulfate). The layers were separated. The aqueous layer was extracted three times with ethyl acetate. The reaction mixture was stirred at this temperature for 20 hours. Ethyl acetate and water were added.

Subsequently, trifluoromethanesulfonic anhydride (0.87 mL, 5.2 mmol) was added dropwise and the suspension of 1,5-dimethylisoquinolin-6-ol (0.60 g, 3.5 mmol) in acetonitrile (66 mL) at 0 °C.

1,5-Dimethylisoquinolin-6-yl trifluoromethanesulfonate 76.28, H: 6.40, N: 8.09; found C: 76.00, H: 6.47, N: 8.21.

1H NMR (500 MHz, methanol-d4): \( \delta = 7.60 \) (d, \( J = 6.2 \) Hz, 1H), 7.43 (d, \( J = 6.2 \) Hz, 1H), 7.37 (d, \( J = 9.2 \) Hz, 1H), 7.23 (d, \( J = 6.2 \) Hz, 1H), 7.13 (s, 1H), 7.03 (d, \( J = 6.2 \) Hz, 1H), 6.93 (d, \( J = 6.2 \) Hz, 1H); 13C NMR (125 MHz, CDCl3): \( \delta = 131.82 \) (CH), 127.66 (C), 123.46 (C), 120.29 (CH), 119.80 (C), 118.81 (C); MS (EI): m/z (%): = 187 (100, [M]+), 174 (100), 158 (23), 144 (23), 131 (22), 115 (21), 105 (21), 91 (22), 77 (29), 63 (17), 51 (20); HRMS: calcd for C12H10NO: 189.0962, found: 189.0958; elemental analysis: calcd for C12H9NO: C: 78.94, H: 6.77, N: 7.32; found C: 78.96, H: 6.86; N: 7.28.

For larger amounts: Freshly distilled hydrobromic acid (22 mL, 0.19 mol) was carefully added at 0 °C to 6-methoxy-1,5-dimethylisoquinoline (13, 3.01 g, 16.1 mmol). After the addition was completed, the cooling bath was removed and the mixture was heated at refluX for five hours. Then, the excess of hydrobromic acid was removed under vacuo. The brownish raw material was completely dissolved in water (115 mL, ultrasound), filtered, and neutralized by dropwise addition of a saturated aqueous solution of sodium bicarbonate. The layers were separated and the aqueous layer was completely evaporated to give 1,5-dimethylisoquinolin-6-ol (40 mg, 0.23 mmol, 96%) as a brownish solid.

1,5-Dimethylisoquinolin-6-ol. For small amounts: In a microwave tube, a mixture of 6-methoxy-1,5-dimethylisoquinoline (13, 45 mg, 0.24 mmol) and pyridinium chloride (1 g, 8 mmol) was irradiated at 155 °C (300 Watt) for 30 minutes. After cooling to room temperature, the mixture was dissolved in water and ethyl acetate, and neutralized with a saturated aqueous solution of sodium bicarbonate. The layers were separated and the aqueous layer was completely evaporated to give 1,5-dimethylisoquinolin-6-ol (40 mg, 0.23 mmol, 96%) as a brownish solid.

For larger amounts: Freshly distilled hydrobromic acid (22 mL, 0.19 mol) was carefully added at 0 °C to 6-methoxy-1,5-dimethylisoquinoline (13, 13.0 g, 64.7 mmol). After the addition was completed, the cooling bath was removed and the mixture was heated at refluX for five hours. Then, the excess of hydrobromic acid was removed under vacuo. The brownish raw material was completely dissolved in water (115 mL, ultrasound), filtered, and neutralized by dropwise addition of a saturated aqueous solution of sodium bicarbonate. The resulting solid was carefully washed with water and dried to provide 1,5-dimethylisoquinolin-6-ol (290 mg, 1.6 mmol, 96%) as a brownish solid.

1,5-Dimethylisoquinolin-6-yl trifluoromethanesulfonate 130.29, C: 76.16, H: 7.99, N: 7.40; found C: 76.25, H: 7.98, N: 7.46.

6-Methoxy-1,5-dimethylisoquinoline (13). A flask filled with 6-methoxy-1,5-dimethyl-3,4-dihydroisoquinoline (90.3 mg, 0.48 mmol) and palladium on charcoal (10%, 93.6 mg) was evacuated under vigorous stirring for 15 min and then filled with argon. Toluene (3.6 mL) and cyclohexene (1.3 mL, 13 mmol) were added and the mixture was heated at refluX until full conversion was detected (TLC: Alox N; ethyl acetate/isohexane, 1:1) to provide 6-methoxy-1,5-dimethylisoquinoline (13, 90 mg, 0.48 mmol, 100%) as a beige solid. M.p. 99–102 °C; UV (MeOH): \( \lambda = 203, 236, 301 \) nm; IR (ATR): \( \nu = 3058, 3015, 2965, 2940, 2847, 1608, 1563, 1542, 1495, 1457, 1401, 1344, 1324, 1267, 1179, 1153, 1116, 1078, 1009, 984, 913, 848, 814, 774, 698, 673, 648, 581, 528 \) cm\(^{-1}\); \( 1^H \) NMR (600 MHz, CDCl3): \( \delta = 7.02 \) (s, 3H), 6.92 (s, 1H), 4.00 (s, 3H), 3.75 (d, \( J = 9.2 \) Hz, 1H), 3.63 (d, \( J = 6.2 \) Hz, 1H), 8.05 (d, \( J = 9.2 \) Hz, 1H), 8.32 (d, \( J = 6.2 \), 1H); \( 1^3C \) NMR (150 MHz, CDCl3): \( \delta = 109.49 \) (CH), 22.17 (CH3), 56.39 (CH3), 111.27 (CH), 115.83 (CH), 118.97 (C), 123.04 (C), 125.66 (CH), 127.47 (C), 136.96 (C), 140.72 (CH), 158.57 (C); MS (EI): m/z (%): = 187 (100, [M]+), 172 (26), 156 (19), 144 (30), 128 (19), 115 (43), 103 (21), 89 (11), 77 (30), 63 (24), 51 (22); HRMS: calcd for C12H10NO: 187.0997, found: 187.0986; elemental analysis: calcd for C12H9NO: C: 76.98, H: 7.00, N: 7.48; found C: 76.43, H: 7.04, N: 7.53.
1,5-Dimethyl-N-phenylisoquinolin-6-amine (16). Aniline (15, 0.1 mL, 1.2 mmol) was added dropwise to a solution of 1,5-dimethylisoquinolin-6-yl trifluoromethanesulfonate (14, 0.235 g, 0.770 mmol), palladium(II) acetate (13 mg, 0.064 mmol) and cesium carbonate (0.35 g, 1.1 mmol) in toluene (20 mL). The mixture was heated at reflux for 48 hours. After cooling to room temperature, the reaction mixture was filtered over a short pad of Hyflo (ethyl acetate) and the solvent was evaporated. Purification of the residue by column chromatography (silica gel, dichloromethane/ethyl acetate 1:3 + 1% methanol) provided 1,5-dimethylisoquinolin-6-yl trifluoromethanesulfonate (15a, 0.19 g, 0.77 mmol, 100%) as a yellow solid. M.p. 175 °C (decomp.); UV (MeOH): \( \lambda = 223, 250, 280, 325, 358 \) (sh) nm; IR (ATR): \( \nu = 3088, 3031, 2995, 2927, 2856, 1612, 1564, 1522, 1473, 1459, 1414, 1375, 1350, 1245, 1207, 1170, 1103, 993, 861, 826, 715, 677, 663, 621 \) cm\(^{-1}\); \( ^{1} \)H NMR (500 MHz, CDCl\(_3\)): \( 6 = 2.74 \) (s, 3H), 6.85 (d, \( J = 9.3 \) Hz, 1H), 7.11 (d, \( J = 6.1 \) Hz, 1H); \( ^{13} \)C NMR (125 MHz, CDCl\(_3\)): \( \delta = 12.41 \) (CH), 22.95 (CH), 116.23 (CH), 118.77 (q, \( J_{CF} = 321 \) Hz, CF\(_3\)), 120.75 (CH), 126.42 (CH and C), 126.64 (C), 136.97 (C), 143.40 (CH), 147.91 (C), 159.50 (C); \( ^{19} \)F NMR (282 MHz, CDCl\(_3\)): \( \delta = -73.58 \) (s, 3F); MS (EI): \( m/z = 248.1313, \) found: 248.1310.

N-(2-Chlorophenyl)-1,5-dimethylisoquinolin-6-amine (18a). 2-Chloroaniline (17a, 78 \( \mu \)L, 0.74 mmol) was added dropwise to a solution of 1,5-dimethylisoquinolin-6-yl trifluoromethanesulfonate (14, 0.15 g, 0.49 mmol), palladium(II) acetate (8.3 mg, 37 \( \mu \)mol), XPhos (55 mg, 0.12 mmol) and cesium carbonate (0.74 g, 2.2 mmol) in toluene (12 mL). The mixture was heated at reflux for 1.5 hours. After cooling to room temperature, the reaction mixture was filtered over a short pad of Hyflo (ethyl acetate) and the solvent was evaporated. Purification of the residue by column chromatography (silica gel, dichloromethane/ethyl acetate 1:3 + 1% methanol) provided N-(2-chlorophenyl)-1,5-dimethylisoquinolin-6-amine (18a, 0.130 g, 0.460 mmol, 94%) as brownish crystals. M.p. 194–198 °C; UV (MeOH): \( \lambda = 221, 249, 278, 320 \) nm; fluorescence (MeOH): \( \lambda_{ex} = 221, \lambda_{em} = 229 \) (sh), 334 nm; IR (ATR): \( \nu = 3198, 3078, 2955, 2919, 2850, 1589, 1567, 1542, 1501, 1474, 1452, 1396, 1370, 1297, 1259, 1254, 1235, 1207, 1159, 1103, 1058, 1039, 973, 862, 827, 808, 775, 768 \) cm\(^{-1}\); \( ^{1} \)H NMR (500 MHz, CDCl\(_3\)): \( 6 = 2.35 \) (s, 3H), 2.95 (s, 3H), 6.15 (br s, 1H), 6.85 (td, \( J = 7.7, 3.0 \) Hz, 1H), 6.95 (dd, \( J = 8.2, 1.4 \) Hz, 1H), 7.10–7.14 (m, 1H), 7.40 (dd, \( J = 8.0, 1.4 \) Hz, 1H), 7.51 (d, \( J = 9.0 \) Hz, 1H), 7.63 (d, \( J = 6.1 \) Hz, 1H), 7.96 (d, \( J = 9.0 \) Hz, 1H), 8.39 (d, \( J = 6.1 \) Hz, 1H); \( ^{13} \)C NMR (125 MHz, CDCl\(_3\)): \( \delta = 12.58 \) (CH), 122.58 (CH), 115.47 (CH), 116.05 (CH), 120.90 (CH), 121.87 (C), 122.07 (CH), 122.83 (C), 124.52 (C), 124.73 (CH), 127.51 (CH), 129.80 (CH), 136.78 (C), 140.07 (C), 142.19 (C), 158.51 (C); MS (EI): \( m/z = 282.1 (100, \left[M^{+}\right]) \); HRMS (ESI): calcd for C\(_{16}\)H\(_{12}\)ClN\(_{2}\): 282.1120, found: 282.1123.

Crystal data: C\(_{16}\)H\(_{12}\)ClN\(_{2}\), crystal size 0.22 × 0.20 × 0.06 mm\(^3\), \( M = 282.76 \) g mol\(^{-1}\), monoclinic, space group: \( \text{Cc}, a = 11.700(2), b = 9.117(2), c = 14.042(3) \AA, \beta = 110.73(3)^{\circ}, V = 1399.1(5) \AA^3, Z = 4, \rho_{\text{calc}} = 1.342 \) g cm\(^{-3}\), \( \mu = 0.264 \) mm\(^{-1}\), \( T = 198(2) \) K, \( \lambda = 0.71073 \) Å, \( \theta \) range: 3.11–27.00°, 20817 reflections collected.
N-(2-Chloro-5-methoxyphenyl)-1,5-dimethylisoquinolin-6-amine (18b). 2-Chloro-5-methoxyaniline (17b, 92 μL, 0.74 mmol) was added dropwise to a solution of 1,5-dimethylisoquinolin-6-yl trifuoromethanesulfonate (14, 0.15 g, 0.49 mmol), palladium(II) acetate (8.3 mg, 37 μmol), XPhos (35 mg, 74 μmol) and cesium carbonate (224 mg, 0.688 mmol) in toluene (12 mL). The mixture was heated at reflux for five hours. After cooling to room temperature, the reaction mixture was filtered over a short pad of Hyflo (ethyl acetate) and the solvent was evaporated. Purification of the residue by column chromatography (silica gel, dichloromethane/ethyl acetate, 1:1 to 0:1, each + 1% ethanol) provided N-(2-chloro-5-methoxyphenyl)-1,5-dimethylisoquinolin-6-amine (18b, 0.141 g, 0.451 mmol, 92%) as a beige solid. M.p. 135–138 °C; UV (MeOH): λ = 224, 277, 322 nm; fluorescence (MeOH): λex = 224, λem = 301 (sh), 336 nm; IR (ATR): ν = 3416, 3068, 2998, 2929, 2853, 1596, 1508, 1447, 1421, 1383, 1343, 1312, 1287, 1230, 1207, 1170, 1138, 1069, 1027, 924, 820, 732, 671, 640 cm−1; 1H NMR (500 MHz, CDCl3): δ = 2.53 (s, 3H), 2.94 (s, 3H), 3.68 (s, 3H), 6.13 (br s, 1H), 6.40 (dd, J = 8.8, 2.8 Hz, 1H), 6.47 (d, J = 2.8 Hz, 1H), 7.28 (d, J = 8.8 Hz, 1H), 7.53 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 6.1 Hz, 1H), 7.97 (d, J = 9.0 Hz, 1H), 8.39 (d, J = 6.1 Hz, 1H); 13C NMR (125 MHz, CDCl3): δ = 2.51 (s, 3H), 134.3 (C), 136.78 (C), 139.77 (C), 141.06 (CH), 142.38 (C), 158.64 (C), 159.20 (C); MS (EI): m/z (%) = 312 (100, [M]+), 277 (80), 262 (76), 247 (13), 233 (18), 219 (12), 139 (10), 117 (16), 63 (10); MS (ESI, +10 V) m/z = 313.3 [M+H]+.

N-(2-Chloro-4-methoxyphenyl)-1,5-dimethylisoquinolin-6-amine (18c). A solution of 2-chloro-4-methoxyaniline (17c, 127 mg, 0.860 mmol) in toluene (4 mL) was added dropwise to a solution of 1,5-dimethylisoquinolin-6-yl trifuoromethanesulfonate (14, 164 mg, 0.537 mmol), palladium(II) acetate (9 mg, 0.04 mmol), XPhos (38 mg, 81 μmol) and cesium carbonate (224 mg, 0.688 mmol) in toluene (10 mL). The mixture was heated at reflux for one hour. After cooling to room temperature, the reaction mixture was filtered over a short pad of Hyflo (ethyl acetate) and the solvent was evaporated. Purification of the residue by column chromatography (silica gel, dichloromethane/ethyl acetate, 9:1 to 0:1, each + 1% ethanol) provided N-(2-chloro-4-methoxyphenyl)-1,5-dimethylisoquinolin-6-amine (18c, 139 mg, 0.444 mmol, 83%) as a beige solid. M.p. 104–107 °C; UV (MeOH): λ = 226, 255, 318 nm; fluorescence (MeOH): λex = 235, λem = 422 nm; IR (ATR): ν = 3229, 3074, 2993, 2948, 2832, 1731, 1633, 1606, 1562, 1485, 1451, 1436, 1387, 1341, 1308, 1283, 1211, 1182, 1112, 1046, 936, 894, 864, 822, 789, 773, 689, 664 cm−1; 1H NMR (500 MHz, CDCl3): δ = 2.51 (s, 3H), 2.93 (s, 3H), 3.80 (s, 3H), 5.87 (br s, 1H), 6.79 (dd, J = 8.7, 2.8 Hz, 1H), 7.02 (d, J = 2.8 Hz, 1H), 7.09 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 9.1 Hz, 1H), 7.62 (d, J = 6.2 Hz, 1H), 7.90 (d, J = 9.1 Hz, 1H), 8.31 (d, J = 6.2 Hz, 1H); 13C NMR (125 MHz, CDCl3): δ = 2.51 (s, 3H), 224, 118 (CH3), 225, 117 (CH3), 222, 116 (CH3), 221, 115 (CH3), 219 (12), 139 (10), 117 (16), 63 (10); MS (ESI, +10 V) m/z = 312.2 [M+H]+; elemental analysis: calcd for C18H17ClN2O: C: 69.12, H: 5.48, N: 8.96; found C: 69.11, H: 5.39, N: 9.17.

Olivacine (1). N-(2-Chlorophenyl)-1,5-dimethylisoquinolin-6-amine (18a, 20 mg, 71 μmol), palladium(II) acetate (4.8 mg, 21 μmol), tri-tert-butylphosphonium tetrafluoroborate (8.1 mg, 42 μmol) and potassium carbonate (39.1 mg, 0.283 mmol) were dissolved in DMF (0.5 mL). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred for 30 min. After filtration over a short pad of Celite (CH2Cl2), the halogenated solvent was evaporated and the residue was dissolved in ethyl acetate, washed three times with water and then with brine. The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried (sodium sulfate). The solvent was evaporated and the residue was purified by column chromatography (silica gel,
dichloromethane/ethyl acetate, 9:1 to 0:1, each + 5% ethanol) to provide olivacine (I, 12.4 mg, 50.3 μmol, 71%) as brown crystals. M.p. 320–324 °C; UV (MeOH): λ = 223, 237, 275, 285, 292, 327, 342, 374, 391 nm; fluorescence (MeOH): λmax = 285, λmin = 431 nm; IR (ATR): ν = 3058, 2965, 2909, 2765, 1674, 1597, 1479, 1467, 1407, 1334, 1311, 1280, 1252, 1222, 1196, 1150, 1108, 1064, 942, 862, 813, 765, 739, 695, 640 cm–1; 1H NMR (500 MHz, methanol-d4): δ = 2.85 (s, 3H), 3.07 (t, J = 1.1 Hz) and 3.09 (s, 3H), 7.24–7.27 (m, 1H), 7.49–7.54 (m, 2H), 7.89 (d, J = 6.3 Hz, 1H), 8.18 (d, J = 6.3 Hz, 1H), 8.27–8.29 (m, 1H), 8.87 (s, 1H); 13C NMR (125 MHz, methanol-d4): δ = 12.62 (CH2), 22.35 (CH3), 111.86 (CH), 111.42 (C), 116.05 (CH), 116.64 (CH), 120.58 (CH), 122.14 (CH), 123.57 (C), 124.41 (C), 127.25 (C), 128.93 (CH), 134.41 (C), 138.84 (CH), 142.80 (C), 144.34 (C), 160.29 (C); MS (EI): m/z (%) = 246 (100, [M]+), 229 (7), 217 (7), 204 (9), 123 (7); MS (ESI, +10 V) m/z = 247.1 [M+H]+; HRMS (ESI): calc'd for C9H14N2: 246.1157, found: 246.1153; elemental analysis: calc'd for C9H14N2: C: 82.90, H: 5.73, N: 11.37; found C: 83.20, H: 5.81, N: 11.42.

Crystal data: C17H14N2·CH3OH, crystal size 0.45 × 0.12 × 0.07 mm3, M = 278.34 g mol–1, orthorhombic, space group: Pbcn, a = 4.860(1), b = 21.337(5), c = 28.048(6) Å, V = 2908.5(11) Å3, Z = 8, ρcalc = 1.271 g cm–3, μ = 0.080 mm–1, T = 198(2) K, λ = 0.7103 Å, θ range: 3.48–25.40°, 60682 reflections collected, 2656 independent (Rint = 0.0501), 198 parameters. The structure was solved by direct methods and refined by full-matrix least-squares on F2; 1934 reflections observed, R1 = 0.0463, wR2 = 0.1044 [I > 2σ(I)]; maximal residual electron density: 0.204 e Å–3. CCDC 1838729.

8-Methoxylicavine (19b). N-(2-Chloro-5-methoxyphenyl)-1,5-dimethylsiquinolin-6-amine (18b, 14 mg, 45 μmol), palladium(II) acetate (3.0 mg, 13 μmol), tri-tert-butyolphosphium tetrafluoroborate (5.1 mg, 27 μmol) and potassium carbonat (24.7 mg, 0.179 mmol) were dissolved in DMF (0.5 mL). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred for 20 min. After filtration over a short pad of Celite (CH2Cl2), the halogenated solvent was evaporated and the residue was dissolved in ethyl acetate, washed three times with water and then with brine. The aqueous layer was extracted with ethyl acetate and the combined organic layers were dried (sodium sulfate). The solvent was evaporated and the residue was purified by column chromatography (silica gel, dichloromethane/ethyl acetate, 9:1 to 0:1, each + 5% ethanol) to provide 8-methoxylicavine (19b, 8.0 mg, 29 μmol, 65%) as yellow crystals. M.p. 280–283 °C; UV (MeOH): λ = 227, 271, 281, 300, 316, 351 nm; fluorescence (MeOH): λmax = 300, λmin = 430, 515 nm; IR (ATR): ν = 3141, 3046, 2993, 2886, 2821, 2731, 1622, 1595, 1563, 1493, 1472, 1460, 1412, 1388, 1335, 1319, 1297, 1267, 1216, 1197, 1160, 1137, 1099, 1068, 1030, 996, 942, 916, 870, 810, 753 cm–1; 1H NMR (500 MHz, DMSO-d6): δ = 2.79 (s, 3H), 3.01 (s, 3H), 3.89 (s, 3H), 6.85 (dd, J = 8.6, 2.2 Hz, 1H), 7.00 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 6.1 Hz, 1H), 8.23 (d, J = 6.1 Hz, 1H), 8.24 (d, J = 8.6 Hz, 1H), 8.77 (s, 1H), 11.26 (s, 1H); 13C NMR (125 MHz, DMSO-d6): δ = 12.36 (CH3), 22.97 (CH3), 55.32 (CH3), 94.84 (CH), 107.55 (CH), 110.68 (C), 113.55 (CH), 114.78 (CH), 116.19 (C), 122.00 (C), 122.28 (CH), 125.00 (C), 131.74 (C), 139.10 (CH), 140.79 (C), 144.13 (C), 158.26 (C), 159.96 (C); MS (EI): m/z (%): 276 (100, [M]+); 261 (14), 233 (49), 138 (8), 116 (10); MS (ESI, +10 V) m/z = 277.1 [M+H]+; HRMS (ESI): calc'd for C11H14N2O: 276.1263, found: 276.1261.

Crystal data: C9H14N2O·CH3OH, crystal size 0.160 × 0.080 × 0.060 mm3, M = 308.37 g mol–1, orthorhombic, space group: Pbcn, a = 4.9253(3), b = 21.4925(15), c = 29.523(2) Å, V = 3125.3(4) Å3, Z = 8, ρcalc = 1.311 g cm–3, μ = 0.685 mm–1, T = 150(2) K, λ = 0.70173 Å, θ range: 2.993–68.188°, 31382
reflections collected, 2811 independent ($R_{int} = 0.0544$), 230 parameters. The structure was solved by
direct methods and refined by full-matrix least-squares on $F^2$; 2283 reflections observed, $R_I = 0.0387$,
$wR_2 = 0.1001$ [$> 2\sigma (I)$]; maximal residual electron density: 0.221 e Å$^{-3}$. CCDC 1838730.

494 9-Methoxy-4,11b-dimethyl-11bH-pyrido[3,4-c]carbazole (20b, 1.1 mg, 4.0 μmol, 9%), brown oil, less polar
side product. UV (MeOH): $λ = 221, 300, 325$ nm; fluorescence (MeOH): $λ_{max} = 221, λ_{em} = 296, 339$ nm; IR
(ADR): $ν = 3414, 3058, 2924, 2855, 1734, 1655, 1632, 1593, 1535, 1484, 1459, 1437, 1377, 1334, 1276, 1231,
1182, 1149, 1074, 935, 856, 740, 683 cm$–$1; $^{1}H$ NMR (600 MHz, methanol-$d_4$): $δ = 1.62$ (s, 3H), 2.74
(s, 3H), 3.94 (s, 3H), 7.14 (d, $J = 10.0$ Hz, 1H), 7.44 (d, $J = 8.7$ Hz, 1H), 7.79 (d, $J = 6.1$ Hz, 1H), 8.01 (d, $J = 2.5$ Hz, 1H), 8.24
(d, 1H), 8.38 (d, 1H); $^{13}C$ NMR (150 MHz, methanol-$d_4$): $δ = 21.67$ (CH$_3$), 33.43 (CH$_3$), 56.12 (CH$_3$), 58.80 (C), 108.13 (CH), 113.67 (CH), 119.73
(CH), 123.16 (CH), 124.56 (CH), 127.23 (C), 132.78 (C, HMBC), 136.16 (CH), 149.15 (CH), 153.81 (C),
156.57 (C, HMBC), 158.04 (C), 162.22 (C), 186.18 (C); MS (EI): $m/z$ (%) = 276 (100, [M+H]+), 261 (100), 233
(25), 218 (52), 190 (16); MS (ESI, +50 V) $m/z$ = 277.2 [M+H]$^+$.

495 9-Methoxy-4,11b-dimethyl-11bH-pyrido[3,4-c]carbazole (19c, 30.1 mg, 109 μmol, 62%) as a yellow solid. M.p. 273–274°C; UV (MeOH): $λ = 224, 242, 272, 296, 332, 394$ nm; fluorescence (MeOH): $λ_{max} = 296, λ_{em} = 471$ nm; IR (ATR): $ν = 3143,
2914, 1632, 1600, 1485, 1436, 1380, 1330, 1230, 1206, 1175, 1170, 1030, 935, 879, 862, 838,
809, 767, 735, 698 cm$–$1; $^{1}H$ NMR (500 MHz, DMSO-$d_6$): $δ = 2.80$ (s, 3H), 3.04 (s, 3H), 3.90 (s, 3H), 7.14
(dd, $J = 10.0, 2.5$ Hz, 1H), 7.44 (d, $J = 8.7$ Hz, 1H), 7.79 (d, $J = 6.1$ Hz, 1H), 8.01 (d, $J = 2.5$ Hz, 1H), 8.24
(d, 1H), 8.38 (d, 1H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $δ = 12.80$ (CH$_3$), 23.45
(CH$_3$), 56.11 (CH$_3$), 104.87 (CH), 111.36 (C), 112.00 (CH), 115.18 (CH), 115.71 (CH), 117.04 (CH),
122.00 (C), 123.65 (C), 125.36 (C), 132.64 (C), 135.73 (C), 139.69 (CH), 141.61 (C), 153.78 (C), 159.21 (C);
MS (EI): $m/z$ (%) = 276 (100, [M+H]+), 261 (90), 233 (27), 116 (10); MS (ESI, +10 V) $m/z$ = 277.1 [M+H]$^+$;
HRMS (ESI): calcd for C$_{18}$H$_{16}$N$_2$O: 276.1264, found: 276.1269.

496 9-Methoxyolivacine (19a). N-(2-Chloro-4-methoxyphenyl)-1,5-dimethyisoquinolin-6-amine (18c, 55.0
mg, 176 μmol), palladium(II) acetate (11.8 mg, 53 μmol), tri-tert-butylphosphonium tetrafluoroborate (20.1 mg, 106 μmol) and potassium carbonate (97.2 mg, 0.703 mmol) were
dissolved in DMF (1.4 mL). The reaction mixture was placed in a preheated oil bath at 140°C and
stirred for 35 min. After filtration over a short pad of Celite (CH$_2$Cl$_2$), the halogenated solvent was
removed, the residue was dissolved in ethyl acetate, washed three times with water and then
with brine. The aqueous layer was extracted with ethyl acetate and the combined organic layers
were dried (sodium sulfate). The solvent was evaporated and the residue was purified by column
chromatography (silica gel, dichloromethane/ethyl acetate, 9:1 to 5:1, each + 5% ethanol) to provide
9-methoxyolivacine (19c, 13.5 mg, 51.5 μmol, 84%) as a yellow solid. M.p. 273–274°C; UV (MeOH): $λ = 277.2 \pm [M+H]^+$.
biological testing. M.p. 239 °C; UV (MeOH): A = 239, 301, 317 nm; fluorescence (MeOH): A<sub>ex</sub> = 301, A<sub>em</sub> = 434, 520 nm; IR (ATR): v = 3505, 3279, 3198, 2827, 1660, 1619, 1474, 1433, 1407, 1341, 1190, 1166, 1138, 1102, 840, 800, 722, 633 cm<sup>-1</sup>; ¹H NMR (500 MHz, methanol-<d>): δ = 2.94 (s, 3H), 3.34 (s, 3H), 6.90 (dd, J = 8.5, 2.1 Hz, 1H), 7.01 (d, J = 2.1 Hz, 1H), 8.18 (d, J = 7.0 Hz, 1H), 8.20 (d, J = 8.5 Hz, 1H), 8.37 (d, J = 7.0 Hz, 1H), 9.00 (s, 1H); ¹³C NMR (125 MHz, methanol-<d>): δ = 24.19 (CH), 115.93 (C), 116.93 (CH), 127.12 (C), 123.95 (C), 127.12 (C), 130.23 (C), 134.44 (C), 146.42 (2C), 157.30 (C), 161.11 (C); MS (EI): m/z (%) = 262 (100, [M]+), 180 (10); MS (ESI, +10 V) m/z = 263.1 [M+H]+, 547 [2M+Na]+; HRMS (ESI): calcd for C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O: 262.1106, found: 262.1107.

In conclusion, we have developed a straightforward synthesis of olivacine (1) and four of its oxygenated pyrido[4,3-<b>b</b>]carbazole derivatives via Buchwald–Hartwig coupling of an isoquinolinyl triflate and an ortho-chloroarylamine followed by a Heck-type cyclization. In a test for inhibition of the growth of <i>M. tuberculosis</i> (strain H<sub>37</sub>Rv), 9-methoxyolivacine (19c) proved to be the most active compound with an MIC<sub>90</sub> value of 1.5 μM and a relatively low toxicity for a mammalian cell line. These initial results indicate that the pyrido[4,3-<b>b</b>]carbazoles are a promising class of compounds for our ongoing search for a carbazole-based tuberculosis drug candidate.

4. Conclusions

Supplementary Materials: Copies of the ¹H NMR, ¹³C NMR and 2D NMR spectra.

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Conflicts of Interest: The authors declare no conflict of interest.

References


Sample Availability: Samples of the compounds are not available from the authors.