Supporting Information

Synthesis and Evaluation of Bicyclic Hydroxypyridones as Inhibitors of Catechol O-methyltransferase

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General Procedures.

All commercially available reagents and solvents were used without further purification unless otherwise stated. Automated flash chromatography was performed on an ISCO CombiFlash Rf™ or Biotage Isolera™ using Biotage, ISCO or Agela Flash cartridges with peak detection at 254 nm. Reverse phase purification was accomplished using a Gilson 215 liquid handler equipped with a Phenomenex C18 column (150 x 20 mm I.D., S-5 μm). Peak collection was triggered by UV detection at 214 or 254 nm. ¹H NMR spectra were recorded on a Bruker 400 instrument operating at 400 MHz with tetramethylsilane or residual protonated solvent used as a reference. Analytical LC-MS was performed using Agilent 1260 equipped with autosampler (Agilent Poroshell 120 C18 column (50 x 3.0 mm I.D., 2.7 μm); 0.05% TFA in water/acetonitrile gradient; UV detection at 215 and 254 nm) and electrospray ionization. All final compounds showed purity greater than 95% at 215 and 254 nm using this method. High-resolution mass spectral data was acquired from m/z 50 – 400 using an Agilent 6540 QTOF with a Jet Stream Ion Source in 4 GHz high resolution mode. Test articles were prepared in 0.1% formic acid in methanol and infused at 0.1 mL min⁻¹. The data was analyzed using Agilent Masshunter Qualitative Analysis Software (B.07.00 SP2).
Compound Synthesis and Characterization Data:

7-benzyloxy-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (15a)

Step 1: preparation of 5-benzyloxy-2-(hydroxymethyl)pyran-4-one (11a)

To a solution of kojic acid (10) (5.00 g, 35.2 mmol) and sodium hydroxide (1.55 g, 38.7 mmol) in methanol (70 mL) and water (7 mL) was added benzyl chloride (4.70 mL, 40.5 mmol) dropwise over 10 min. The resulting mixture was stirred at reflux for 2 h, then overnight at room temperature. After the solvent volume was reduced roughly in half in vacuo, water was added and the mixture extracted with EtOAc (2 x 200 mL). The EtOAc layers were combined, washed with water (3 x 100 mL), brine (50 mL), dried over MgSO4 and filtered. As the solvent was being removed in vacuo, a crystalline solid began to form. This material was collected by filtration, washed with EtOAc and air-dried to give 5-benzyloxy-2-(hydroxymethyl)pyran-4-one (4.35 g, 18.7 mmol, 53.2% yield) as an off-white solid. This material was used without further purification. MS, ES⁺ m/z 233.0 [M+H]+.

1H-NMR (400 MHz, DMSO-d6) δ 8.19 (s, 1 H) 7.33 - 7.44 (m, 5 H) 6.33 (s, 1 H) 5.70 (t, J = 6.19 Hz, 1 H) 4.94 (s, 2 H) 4.30 (d, J = 6.06 Hz, 2 H)

Step 2: preparation of 1-(2-aminoethyl)-5-benzyloxy-2-(hydroxymethyl)pyridin-4-one (12a)
Ethylene diamine (45.3 g, 754 mmol, 50.9 mL) was added to a suspension of 5-(benzyloxy)-2-(hydroxymethyl)-4H-pyran-4-one (11a) (35 g, 151 mmol) in ethanol (350 mL). The mixture was heated at 90°C for 1.5 h. The reaction mixture was concentrated to yield 1-(2-aminoethyl)-5-benzyloxy-2-(hydroxymethyl)pyridin-4-one (12a) as a brown viscous oil (46.4 g, quant.), which was used as such in the next experiment without further purification. MS, ES+ m/z 275.2 [M+H]+.

1H-NMR (400 MHz, DMSO-d6) δ 7.54 (s, 1 H) 7.30 - 7.44 (m, 5 H) 6.22 (s, 1 H) 5.00 (s, 2 H) 4.39 (s, 2 H) 3.84 (t, J = 6.19 Hz, 2 H) 2.80 (t, J = 6.06 Hz, 2 H)

Step 3: preparation of 7-benzyloxy-3,4-dihydropyrido[1,2-a]pyrazin-8-one (13a)

1-(2-aminoethyl)-5-benzyloxy-2-(hydroxymethyl)pyridin-4-one (12a) (49 g, 179 mmol) was dissolved in CHCl₃ (400 mL) and MnO₂ (78 g, 893 mmol) was added to the mixture which was heated overnight at 60°C. The reaction mixture was filtered and the residue was concentrated to yield 7-benzyloxy-3,4-dihydropyrido[1,2-a]pyrazin-8-one (13a), which was used in the next step without further purification. MS, ES+ m/z 255.5 [M+H]+.

Step 4: preparation of 7-benzyloxy-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14a)

7-benzyloxy-3,4-dihydropyrido[1,2-a]pyrazin-8-one (13a) (46.4 g, 182 mmol) was dissolved in MeOH (500 mL). NaBH₄ (20.71 g, 547 mmol) was added portionwise to the mixture and the mixture was stirred over 48 h at room temperature. The reaction mixture was then concentrated to give a residue (10 g) that was purified by chromatography (SiO₂, DCM:MeOH:NH₃ (7 N) (90:9:1) to yield 7-benzyloxy-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14a) as a yellow solid (23.4 g, 50%). MS, ES+ m/z 257.2 [M+H]+.

1H-NMR (300 MHz, DMSO-d6) δ 7.45-7.22 (m, 6H), 5.90 (s, 1H), 4.96 (s, 2H), 3.74 (t, J = 5.6 Hz, 2H), 3.70 (br.s., 2H), 3.00 (t, J = 5.6 Hz, 2H), 2.57 (br.s., 1H), 1H-NMR (300 MHz, MeOD) δ 7.48-7.42 (m, 3H), 7.38-7.26 (m, 3H), 6.25 (s, 1H), 5.06 (s, 1H), 3.97-3.81 (m, 4H), 3.28 (t, J = 5.6 Hz, 2H).
7-[(4-methoxyphenyl)methoxy]-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14b)

Step 1: preparation of 2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)-4H-pyran-4-one (11b)

To a suspension of kojic acid (10) (25.4 g, 179 mmol) in anhydrous DMF (450 mL) was added potassium carbonate (29.6 g, 214 mmol) and alpha-chloro-4-methoxytoluene (30.8 g, 197 mmol, 26.7 mL) and the resulting suspension was stirred at 80°C for 4 h. The reaction mixture was evaporated in vacuo (oil pump, high vacuum) and water (500 mL) was added. The resulting suspension was stirred for 30 minutes. The solids were filtered off and dried under air current for 30 minutes, then triturated from heptane:diethyl ether (1:1, 300 mL) and dried in a vacuum oven (40°C) overnight to yield 38.44 g of 2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)-4H-pyran-4-one (11a) as a brown solid, which was used in the next step without any further purification.

Step 2: preparation of 1-(2-aminoethyl)-2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)pyridin-4(1H)-one (12b)

Ethylenediamine (38.2 g, 635 mmol, 42.9 mL) was added to a suspension of 2-hydroxymethyl)-5-((4-methoxybenzyl)oxy)-4H-pyran-4-one (11b) (33.3 g, 127 mmol) in Ethanol (400 mL). The reaction mixture was stirred for 4 h at 90°C and then stirred overnight at room temperature. The reaction mixture was concentrated to yield 1-(2-aminoethyl)-2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)pyridin-4(1H)-one.
(12b) (50.3 g) as a brown viscous oil which was used in the next step without any further purification. MS,
ES+ m/z 305.2 [M+H]+.

**Step 3: Preparation of 7-((4-methoxybenzyl)oxy)-3H-pyrido[1,2-a]pyrazin-8(4H)-one (13b)**

\[
\begin{align*}
&\text{1-(2-aminoethyl)-2-(hydroxymethyl)-5-((4-methoxybenzyl)oxy)pyridin-4(1H)-one (12b) (38.6 g, 127 mmol)} \\
&\text{was dissolved in chloroform (400 mL) and stirred for a few minutes. MnO}_2\text{ (55.1 g, 634 mmol) was added} \\
&\text{to the mixture was stirred at 60 °C overnight. The reaction mixture was filtered and the residue was} \\
&\text{concentrated to yield 7-((4-methoxybenzyl)oxy)-3H-pyrido[1,2-a]pyrazin-8(4H)-one (13b) (47.5 g) and was} \\
&\text{used in the next step without further purification. MS, ES+ m/z 285.2 [M+H]+.}
\end{align*}
\]

**Step 4: preparation of 7-[(4-methoxyphenyl)methoxy]-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14b)**

\[
\begin{align*}
&\text{To a cooled (ice bath) solution of 7-[(4-methoxyphenyl)methoxy]-3,4-dihydropyrido[1,2-a]pyrazin-8-one (13b) (5.0 g, 17.59 mmol) in MeOH (100 mL) was added NaBH}_4\text{ (672 mg, 1 eq.) and the resulting mixture} \\
&\text{was stirred at rt for 14 h. The reaction mixture was filtered on a bed of Celite/silica gel/Celite and rinsed} \\
&\text{with MeOH (3 x 10 mL). The solvent was evaporated to give a pale yellow solid (6.1 g). This solid was} \\
&\text{then dissolved in a DCM/MeOH (9:1). The mixture was then washed with brine. The organic layers were} \\
&\text{dried on MgSO}_4\text{ and concentrated under vacuum to yield 7-[(4-methoxyphenyl)methoxy]-1,2,3,4-} \\
&\text{tetrahydropyrido[1,2-a]pyrazin-8-one (14b) as a yellow solid (4.6 g, 90 %). MS, ES+ m/z 287.2 [M+H]+.}
\end{align*}
\]

\[\text{1H-NMR (300 MHz, DMSO-d}_6\text{) }\delta 7.35 (s, 1H), 7.31 (d, } J = 4.3 \text{ Hz, 2H), 6.91 (d, } J = 4.3 \text{ Hz, 2H), 5.88 (s,} \\
\text{1H), 4.89 (s 2H), 3.75-3.71 (m, 4H), 3.73 (s, 3H), 3.01 (t, } J = 5.9, 2H), \text{ 1H-NMR (300 MHz, MeOH-d}_4\text{) }\delta \\
\text{7.42 (s, 1H), 7.36 (d, } J = 4.3 \text{ Hz, 2H), 6.90 (d, } J = 4.3 \text{ Hz, 2H), 6.25 (s, 1H), 5.05 (s 2H), 3.97-3.92 (m,} \\
\text{4H), 3.78 (s, 3H), 3.19 (t, } J = 5.9, 2H). \text{7-hydroxy-2-[(2-chloro-4-fluorophenyl)methyl]-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one (37)}\]
**Method A**

**Step 1**

To a suspension of 7-[(4-methoxyphenyl)methoxy]-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14b) (175 mg, 0.61 mmol) in DCE (10 mL) was added 2-chloro-4-fluorobenzaldehyde (106.6 mg, 0.67 mmol) and sodium triacetoxyborohydride (388 mg, 1.83 mmol). The resulting mixture was stirred at room temperature for 18 h. The contents were treated with 1 N NaOH, added 10% Na₂CO₃ and extracted with CHCl₃ (3x). The organic layers were combined, dried over Na₂SO₄, filtered and the solvent removed in vacuo to give a residue which was purified by automated normal-phase chromatography (0-30% MeOH/DCM, silica gel) to give 2-[(2-chloro-4-fluorophenyl)methyl]-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (153 mg, 0.357 mmol, 58 % yield) as a white foam. MS, ES⁺ m/z 429.3 [M+H]⁺.

**Step 2**

To a solution of 2-[(2-chloro-4-fluorophenyl)methyl]-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (149 mg, 0.35 mmol) in EtOH (5 mL) was added 6 M HCl (3.16 mL, 18.95 mmol). The resulting mixture was stirred at 100 °C for 3 h, then allowed to reach room temperature with stirring overnight (for convenience). The solvent was removed in vacuo and the residue was triturated with Et₂O (3x) and the triturants removed by decantation. The solid was dried under vacuum to give 2-[(2-chloro-4-fluoro-phenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one hydrochloride (111 mg, 0.322 mmol, 92.5 % yield) as an off-white solid. MS, ES⁺ m/z 309.0 [M+H]⁺.

¹H NMR (400 MHz, DMSO-d₆) δ ppm 11.09 - 11.38 (m, 1 H) 8.10 (s, 1 H) 7.61 - 7.67 (m, 1 H) 7.51 (dd, 1 H) 7.29 (td, 1 H) 7.15 (s, 1 H) 4.43 (t, 2 H) 4.02 (br. s., 2H) 3.91 (br. s., 2H) 3.10 (br. s., 2 H)
The following compounds (as their hydrochloride salts) have been synthesized according to **Method A**

| Ex. | Name | Structure | Analytical data | Preparation Information |
|-----|------|-----------|-----------------|-------------------------|
| 16  | 7-hydroxy-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one | ![Structure](image1) | MS, ES+, 167.2 (M+H)+, <br>\(^1\)H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 10.19 (br. s., 1 H) 8.15 (s, 1 H) 7.15 (br. s., 1 H) 4.45 - 4.55 (m, 4 H) 3.59 (d, J = 5.3 Hz, 2 H) | **Method A**, (step 2 only), using 14b |
| 19  | 2-benzyl-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image2) | MS, ES+, 257.2 (M+H)+. <br>High-Res MS for C<sub>15</sub>H<sub>17</sub>N<sub>2</sub>O<sub>2</sub>+, ES+, 257.1286 (M+H)+ (obs.), 257.1285 (M+H)+ (calc.). <br>\(^1\)H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 11.35 (br. s., 1 H) 8.20 (s, 1 H) 7.24 (s, 1 H) 4.59 (br. s., 2 H) 4.33 (br. s., 2 H) 4.26 (br. s., 2 H) 3.44 (br. s., 2 H) | **Method A** using benzaldehyde and 14b |
| 21  | 7-hydroxy-2-(3-pyridylmethyl)-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image3) | MS, ES+, 258.0 (M+H)+, <br>\(^1\)H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 11.38 (br. s., 1 H) 8.97 (s, 1 H) 8.91 (d, J = 4.80 Hz, 1 H) 8.61 (d, J = 8.08 Hz, 1 H) 8.19 (s, 1 H) 8.06 (dd, J = 7.96, 5.43 Hz, 1 H) 7.21 (s, 1 H) 4.48 (t, J = 5.31 Hz, 2 H) 4.16 (br. s., 2 H) 4.12 (br. s., 2 H) 3.22 (br. s., 2 H) | **Method A** using 3-pyridinecarboxaldehyde and 14b |
| 24  | 2-[(2-cyanophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image4) | MS, ES+, 282.0 (M+H)+, <br>\(^1\)H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 11.21 (br. s., 1 H) 8.09 (s, 1 H) 7.87 - 7.90 (m, 1 H) 7.71 - 7.77 (m, 1 H) 7.65 - 7.69 (m, 1 H) 7.51 - 7.57 (m, 1 H) 7.14 (s, 1 H) 4.40 (t, J = 5.18 Hz, 2 H) 3.96 (br. s., 2 H) 3.94 (br. s., 2 H) 3.04 (t, J = 5.31 Hz, 2 H) | **Method A** using 2-cyanobenzaldehyde and 14b |
| Ex. | Name | Structure | Analytical data | Preparation Information |
|-----|------|-----------|-----------------|-------------------------|
| 25  | 2-[(4-cyanophenyl)met hyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES⁺, 282.0 (M+H)⁺, ¹H NMR (400 MHz, DMSO-d₆) δ ppm 8.01 (s, 1 H) 7.84 - 7.88 (m, 2 H) 7.58 (d, J = 8.3 Hz, 2 H) 6.99 (s, 1 H) 4.37 (t, J = 5.3 Hz, 2 H) 3.83 (s, 2 H) 2.96 (t, J = 5.3 Hz, 2 H) | Method A using 4-cyanobenzaldehyde and 14b |
| 26  | 7-hydroxy-2-[(2-methoxyphenyl)m ethyl]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES⁺, 287.0 (M+H)⁺, ¹H NMR (400 MHz, DMSO-d₆) δ ppm 7.94 (s, 1 H) 7.31 - 7.40 (m, 2 H) 7.06 (d, J = 7.83 Hz, 1 H) 6.95 - 7.01 (m, 1 H) 6.92 (s, 1 H) 4.35 (t, J = 5.56 Hz, 3 H) 3.97 (s, 2 H) 3.85 (br. s., 2 H) 3.81 (s, 3 H) 3.08 (br. s., 2 H) | Method A using 2-methoxybenzaldehyde and 14b |
| 27  | 7-hydroxy-2-[(4-methoxyphenyl)m ethyl]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES⁺, 287.0 (M+H)⁺, ¹H NMR (400 MHz, DMSO-d₆) δ ppm 7.81 (br. s., 1 H) 7.29 (d, J = 8.59 Hz, 2 H) 6.94 (d, J = 8.84 Hz, 2 H) 6.73 (br. s., 1 H) 4.25 (br. s., 2 H) 3.80 (br. s., 2 H) 3.76 (s, 3 H) 3.70 (d, J = 3.28 Hz, 2 H) 2.95 (br. s., 2 H) | Method A using 4-methoxybenzaldehyde and 14b |
| 28  | 7-hydroxy-2-[(2-(trifluoromethyl)phenyl)m ethyl]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES⁺, 325.0 (M+H)⁺, ¹H NMR (400 MHz, DMSO-d₆) δ ppm 11.26 (br. s., 1 H) 8.12 (s, 1 H) 7.84 (d, J = 7.58 Hz, 1 H) 7.77 (d, J = 7.83 Hz, 1 H) 7.71 (t, J = 7.71 Hz, 1 H) 7.51 - 7.57 (m, 1 H) 7.16 (s, 1 H) 4.42 (t, J = 5.31 Hz, 2 H) 3.98 (br. s., 2 H) 3.94 (br. s., 2 H) 3.03 (br. s., 2 H) | Method A using 2-trifluoromethylbenzaldehyde and 14b |
| 29  | 7-hydroxy-2-[(4-(trifluoromethyl)phenyl)m ethyl]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES⁺, 325.0 (M+H)⁺, ¹H NMR (400 MHz, DMSO-d₆) δ ppm 8.16 (s, 1 H) 7.77 - 7.82 (m, 2 H) 7.70 - 7.74 (m, 2 H) 7.19 (s, 1 H) 4.50 (br. s., | Method A using 4-trifluoromethylbenzaldehyde and 14b |
| Ex. | Name | Structure | Analytical data | Preparation Information |
|-----|------|-----------|-----------------|--------------------------|
| 31  | 2-[(2-chlorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image1) | MS, ES⁺, 291.2 (M+H)⁺,  
High-Res MS, ES⁺, 291.0905 (M+H)⁺ (obs.), 291.0894 (M+H)⁺ (calc. for C₁₅H₁₆ClN₂O₂⁺).  
¹H NMR (400 MHz, DMSO-d₆) δ ppm  11.34 (br. s., 1 H)  
8.17 (s, 1 H) 7.68 (br. s., 1 H)  
7.50 - 7.56 (m, 1 H) 7.38 - 7.45 (m, 2 H) 7.24 (s, 1 H)  
4.50 (br. s., 2 H) 4.19 (br. s., 2 H) 3.26 (br. s., 2 H) | Method A using 2-chlorobenzaldehyde and 14b |
| 33  | 2-[(2,4-dimethylphenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image2) | MS, ES⁺, 285.0 (M+H)⁺,  
¹H NMR (400 MHz, DMSO-d₆) δ ppm  11.30 (br. s., 1 H)  
8.16 (s, 1 H) 7.37 (br. s., 1 H)  
7.22 (s, 1 H) 7.03 - 7.10 (m, 2 H) 4.54 (br. s., 2 H) 4.26 (br. s., 2 H) 4.10 (br. s., 2 H) 3.37 (br. s., 2 H) 2.38 (s, 3 H) 2.29 (s, 3 H) | Method A using 2,4-dimethylbenzaldehyde and 14b |
| 35  | 2-[(2,6-dimethylphenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one hydrochloride | ![Structure](image3) | MS, ES⁺, 285.2 (M+H)⁺,  
¹H NMR (400 MHz, DMSO-d₆) δ ppm  11.31 (br. s., 1 H)  
8.14 (br. s., 1 H) 7.05 - 7.29 (m, 4 H) 4.46 (br. s., 2 H) 4.04 (br. s., 3 H) 3.14 (br. s., 1 H) 2.42 (br. s., 6 H) | Method A using 2,6-dimethylbenzaldehyde and 14b |
| 38  | 2-[(2,4-dichlorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image4) | MS, ES⁺, 325.0 (M+H)⁺,  
¹H NMR (400 MHz, DMSO-d₆) δ ppm 11.26 (br. s., 1 H) | Method A using 2,4-dichlorobenzaldehyde and 14b |
| Ex. | Name                                                                 | Structure                                                                 | Analytical data                                                                 | Preparation Information                           |
|-----|----------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------|
| 39  | 2-[(2,6-difluorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure Image](image1)                                                   | MS, ES+, 293.1 (M+H)†                                                             | Method A using 2,6-difluorobenzaldehyde and 14b   |
| 40  | 2-[(2-chloro-6-fluorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure Image](image2)                                                   | MS, ES+ 309.0 (M+H)*, High-Res MS, ES+, 309.0808 (M+H)* (obs.), 309.0801 (M+H)* (calc. for C_{15}H_{15}ClFNO_{2}+) | Method A using 2-chloro-4-fluorobenzaldehyde and 14b |
| 41  | 2-[(2,6-dichlorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure Image](image3)                                                   | MS, ES+, 325.0 (M+H)*                                                             | Method A using 2,6-dichlorobenzaldehyde and 14b   |

2-(2-ethylbutyl)-7-hydroxy-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one (18)
Method B

0.6 mL of THF, 0.3 mL of EtOH and 0.1 mL of HOAc were added to 7-benzyloxy-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (14a, 19.2 mg, 0.075 mmol). 2-ethylbutryaldehyde (11.3 mg, 13.8 µL, 1.5 eq.) followed by Si-BH3CN (Silicycle, 250 mg, 0.223 mmol, 3 eq.) were added. The resulting reaction mixture was stirred overnight at room temperature. The mixture was filtered, evaporated. The residue was then dissolved in CH3CN (2 mL) and purified by reverse phase preparative chromatography.

An aq. solution of HCl (1 mL, 6 N) was added to the purified and evaporated fractions and the mixture was heated at 100°C for 1.5 h. The solvent was evaporated under vacuum. The residue was dissolved in CH3CN / H2O 75 : 25 (2 mL) and the solvents evaporated under vacuum to yield 2-(2-ethylbutyl)-7-hydroxy-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one as a colorless film (16.9 mg). High-Res MS, ES+, 251.1755 (M+H)+ (obs.), 251.1759 (M+H)+ (calc. for C14H23N2O2+). 1H NMR (400 MHz, DMSO-d6) δ ppm 8.17 (s, 1 H) 7.19 (s, 1 H) 4.61 (br. s., 2 H) 3.38 - 3.83 (m, 4 H) 2.83 - 3.02 (m, 2 H) 1.75 (br. s., 1 H) 1.41 (td, J = 14.65, 7.07 Hz, 4 H) 0.86 (t, J = 7.45 Hz, 6 H).

The following compounds have been synthesized according to Method B

| Ex. | Name | Structure | Analytical data | Preparation Information |
|-----|------|-----------|-----------------|-------------------------|
| 17  | 7-hydroxy-2-isobutyl-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | | MS, ES+, 223.1 (M+H)+, 1H NMR (400 MHz, DMSO-d6) δ ppm 8.19 (s, 1 H) 7.21 (s, 1 H) 4.63 (br. s., 2 H) 3.55 (br. s., 4 H) 2.91 (br. s., 2 H) 2.09 (br. s., 1 H) 1.00 (d, J = 6.57 Hz, 6 H) | Method B using 2-methylpropanal and 14a |
| 20  | 7-hydroxy-2-(2-phenylethyl)-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | | MS, ES+, 271.1 (M+H)+, 1H NMR (400 MHz, DMSO-d6) δ ppm 8.14 (s, 1 H) 7.24 - 7.38 (m, 5 H) 7.14 (s, 1 H) 4.59 (br. s., 2 H) 3.75 - 3.95 (m, 2 H) 3.58 (br. s., 2 H) 3.16 - 3.35 (m, 2 H) 3.05 (br. s., 2 H) | Method B using phenylacetaldehyde and 14a |
| Ex. | Name | Structure | Analytical data | Preparation Information |
|-----|------|-----------|-----------------|-------------------------|
| 30  | 7-hydroxy-2-(2-methylbenzyl)-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES<sup>+</sup>, 271.0 (M+H)<sup>+</sup>, 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.14 (s, 1 H) 7.45 (br. s., 1 H) 7.22 - 7.31 (m, 3 H) 7.20 (s, 1 H) 4.51 (br. s., 2 H) 4.17 (br. s., 2 H) 4.02 (br. s., 2 H) 2.40 (s, 3 H) | Method B using 2-methylbenzaldehyde and 14a |
| 32  | 2-[(2,3-dimethylphenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES<sup>+</sup>, 285.0 (M+H)<sup>+</sup>, 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.11 (s, 1 H) 7.08 - 7.21 (m, 4 H) 4.47 (br. s., 2 H) 4.12 (br. s., 2 H) 3.93 - 4.03 (m, 2 H) 3.17 - 3.27 (m, 2 H) 2.27 (s, 6 H) | Method B using 2,3-dimethylbenzaldehyde and 14a |
| 34  | 2-[(2,5-dimethylphenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES<sup>+</sup>, 285.1 (M+H)<sup>+</sup>, 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.10 (s, 1 H) 7.04 - 7.19 (m, 4 H) 4.47 (br. s., 2 H) 4.04 - 4.16 (m, 2 H) 3.92 - 4.02 (m, 2 H) 3.17 (br. s., 2 H) 2.33 (s, 3 H) 2.28 (s, 3 H) | Method B using 2,5-dimethylbenzaldehyde and 14a |
| 36  | 2-[(2-fluorophenyl)methyl]-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one | ![Structure](image) | MS, ES<sup>+</sup>, 275.0 (M+H)<sup>+</sup>, 1H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 8.15 (s, 1 H) 7.61 (l, J = 7.33 Hz, 1 H) 7.42 - 7.50 (m, 1 H) 7.25 - 7.32 (m, 2 H) 7.22 (s, 1 H) 4.50 (br. s., 2 H) 4.17 (br. s., 2 H) 4.09 (br. s., 3 H) 3.27 (br. s., 2 H) | Method B using 2-fluorobenzaldehyde and 14a |

2-(4-fluorobenzoyl)-7-hydroxy-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one (22)
**Step 1**

A suspension of 7-[(4-methoxyphenyl)methoxy]-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (75 mg, 0.26 mmol), 4-fluorobenzoic acid (35.4 µL, 0.29 mmol), HBTU (109.3 mg, 0.29 mmol) and DIPEA (137 µL, 0.79 mmol) in chloroform (5 mL) was stirred at room temperature for 18 h. The contents were treated with 5% Na2CO₃ and extracted with CHCl₃ (3x). The organic layers were filtered through a cotton plug, reduced in volume with a stream of N₂ and then purified by automated normal-phase chromatography (0-30% MeOH/DCM, 4 g silica gel cartridge) to give 2-(4-fluorobenzoyl)-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (72.8 mg, 0.178 mmol, 68.1% yield) as a tan solid. MS, ES⁺ m/z 409.0 [M+H]⁺.

**Step 2**

To a suspension of 2-(4-fluorobenzoyl)-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (84.7 mg, 0.210 mmol) in Ethanol (5 mL) was added 6 M HCl (0.69 mL, 4.15 mmol). The resulting mixture was stirred at 80 °C for 2 h. After cooling, the solvent was removed in vacuo and the residue triturated with Et₂O (3x). The precipitate was collected by filtration, washed with Et₂O and dried under vacuum to give 2-(4-fluorobenzoyl)-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one hydrochloride (47.7 mg, 0.147 mmol, 70.8% yield) as a white solid.

MS, ES⁺ m/z 289.0 [M+H]⁺.

¹H NMR (400 MHz, DMSO-d₆) δ ppm 8.22 (s, 1 H) 7.56 - 7.63 (m, 2 H) 7.31 - 7.38 (m, 3 H) 4.90 (br. s., 2 H) 4.43 - 4.53 (m, 2 H) 3.55 - 3.61 (m, 2 H)

2-benzenesulfonyl-7-hydroxy-1,2,3,4-tetrahydro-8H-pyrido[1,2-a]pyrazin-8-one (23)
Step 1

To a suspension of 7-[(4-methoxyphenyl)methoxy]-1,2,3,4-tetrahydropyrido[1,2-a]pyrazin-8-one (78 mg, 0.27 mmol) and DIPEA (142.4 µL, 0.82 mmol) in Chloroform (5 mL) was added benzenesulfonyl chloride (41.8 µL, 0.33 mmol). The resulting mixture was stirred at room temperature for 30 min. The contents were treated with 5% Na₂CO₃ and extracted with CHCl₃ (3x). The organic layers were filtered through a cotton plug, reduced in volume with a stream of N₂ and then purified by automated normal-phase chromatography (0-30% MeOH/DCM, 4 g silica gel cartridge) to give 2-(benzenesulfonyl)-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (61.7 mg, 0.145 mmol, 53.1 % yield) as a tan foam. MS, ES⁺ m/z 427.0 [M+H]⁺.

Step 2

To a suspension of 2-(benzenesulfonyl)-7-[(4-methoxyphenyl)methoxy]-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one (61.7 mg, 0.14 mmol) in Ethanol (5 mL) was added 6 M HCl (0.48 mL, 2.89 mmol). The resulting mixture was stirred at 80 °C for 2 h. After cooling, the solvent was removed in vacuo and the residue triturated with Et₂O (3x). The precipitate was collected by filtration, washed with Et₂O and dried under vacuum to give 2-(benzenesulfonyl)-7-hydroxy-3,4-dihydro-1H-pyrido[1,2-a]pyrazin-8-one hydrochloride (35.8 mg, 0.104 mmol, 72.2% yield) as a beige solid.

MS, ES⁺ m/z 307.0 [M+H]⁺.

¹H NMR (400 MHz, DMSO-d₆) δ ppm 8.06 (s, 1 H) 7.83 - 7.89 (m, 2 H) 7.73 - 7.79 (m, 1 H) 7.62 - 7.70 (m, 2 H) 7.14 (s, 1 H) 4.52 (s, 2 H) 4.35 - 4.42 (m, 2 H) 3.58 (t, J = 5.31 Hz, 2 H)

ADME assays

Kinetic solubility, hepatocyte stability, and MDCK Pgp efflux assays were performed at Sundia (http://www.sundia.com/site/discovery_dmpk_details).

MB-COMT inhibition assay
from Kimos M, Burton M, Urbain D, Caudron D, Martini M, Famelart M, Gillard M, Barrow J, Wood M. Development of an HTRF Assay for the Detection and Characterization of Inhibitors of Catechol-O-Methyltransferase. *J. Biomol. Screen.* 2016, 21, 490.

**MB-COMT enzyme purification:**
MB-COMT was expressed in HEK293 cells using a pCDNA3.1 vector system and 293 Fectin. Cells (300 mL) at 83% viability were pelleted (15,000 g, 4 °C, 5 min) and resuspended once in phosphate-buffered saline (PBS) and then in membrane buffer (15 mM Tris, pH 7.5, 1 mM EGTA, 0.3 mM EDTA, 2 mM MgCl₂, PIC cocktail [Roche, Indianapolis, IN, ref. 04693159001]). The samples were then homogenized and frozen and defrosted twice in liquid nitrogen. DNase (Sigma-Aldrich, St. Louis, MO, cat no. D4527) was then added (5 µL per mL) and incubated for 10 min at room temperature. The samples were then centrifuged at 40,000 g at 4 °C for 25 min. The supernatant was discarded, and the pellet was resuspended in Tris-sucrose buffer (20 mM Tris, pH 7.4, 250 mM sucrose) and homogenized again. The resulting solution was aliquoted and stored at −80 °C until use. Although the lysate does undergo centrifugation steps, the enzyme concentrations stated herein refer to total protein contained in the extract.

**Assay Procedure:**
An HTRF kit purchased from CisBio was used: EPIgeneous methyltransferase assay, 10,000 tests, cat. 62SAHPEH. Assay reagents were kept frozen at −80 °C, thawed, and used immediately. Stock solutions were made and dispensed into single-use aliquots. No stability information is available longer than 1 day after freeze–thaw.

**Plate:** PerkinElmer Proxi Plate (Waltham, MA), 384 Plus, cat. 6008289

**Compound:** Tolcapone, purchased from Sigma-Aldrich, cat. SML0150
**Assay buffer:** 100 mM NaH$_2$PO$_4$/Na$_2$HPO$_4$, pH 7.4, 10 mM MgCl$_2$, Tween 0.005%, 1 mM DTT

Compounds were diluted in two steps, the first in 100% DMSO (1:32 dilution of 10 mM liquid stock) and then by 20× into assay buffer (5 µL into 95 µL assay buffer using a mosquito dispenser, TTP LabTech, Cambridge, MA), resulting in a final DMSO concentration of 1% (1 µL in 5 µL reaction). Two microliters of a mixture of 10 ng (final concentration per well) of lysate preparation of MB-COMT, premixed with SAM (final concentration of 20 µM, 2 mM stock solution provided by kit), was added to the plate and allowed to incubate with compound for 30 min at 37 °C. Freshly prepared dopamine solution (Sigma H8507) was added to give a reaction concentration of 4 µM, and the reaction was allowed to proceed for 40 min at 37 °C. The DB1, cryptate antibody, and detector were added (no stop buffer used in the MTS format) and incubated according to the kit insert (50× dilution of antibody and 8× dilution of SAH-red based on the 20 µM SAM in the reaction) using a Formulatrix Tempest liquid handler. The results were read on a Tecan Infinite Pro or PerkinElmer Envision and analyzed using Prism GraphPad software.

**Standard curves:**

A standard curve was run on every plate. Using the HTRF ratio (665 nM/620 nM × 10$^4$), a standard curve is determined according to the manufacturer’s instructions using a mixture of SAM and SAH, with the final concentration of SAM being equivalent to that used in the enzyme reaction mixture.

**Determination of Inhibition:**

Tolcapone is known to be a potent inhibitor of COMT and, as such, was used as a benchmark for assay performance. The assays were run under substrate $K_{m,app}$ conditions; the compound was
allowed to preincubate with the enzyme/SAM mixture for 30 min prior to initiating the reaction with dopamine. The MTS assay was designed to be as physiological as possible, so it was run at 37 °C. Tolcapone was potent with an IC$_{50}$ of 1.9 nM ± 1 ($n = 4$, pIC$_{50}$ 8.7). All were run with a 10-point dose–response curve starting at 32 µM. Inhibition of subsequently tested compounds was compared to tolcapone at 10 µM, which was set as the 100% inhibition value.

**S-COMT inhibition assay**

from Buchler, I.; Akuma, D.; Au, V.; Carr, G.; de León, P.; DePasquale, M.; Ernst, G.; Huang, Y.; Kimos, M.; Kolobova, A.; Poslusney, M.; Wei, H.; Swinnen, D.; Montel, F.; Moureau, F.; Wood, M. Optimization of 8-Hydroxyquinolines as Inhibitors of Catechol O-Methyltransferase. *J. Med. Chem.* 2018, 61 (21), 9647–9665.

**S-COMT enzyme purification:**

C-Terminal Hexa-His S-COMT (human and rat) was constructed in a pTT5 mammalian cell expression vector (Invitrogen, Carlsbad, CA) and expressed in 293-6E cells (ATTC, Manassas, VA) using linear polyethylenimine (PEI, MilliporeSigma, St. Louis, MO). Then 0.5 L of cells were harvested at 90% viability and pelleted (15000 g, 4 °C, 5 min). Pellets were frozen at −80 °C. The protein was purified from the pellets using Ni-NTA affinity chromatography and size exclusion per the following: Resin, Ni-NTA (Qiagen, Hilden, Germany) equilibrated in buffer A. Bed volume: 4 mL. Column: 1.0 cm diameter Econo-column (BioRad, Hercules, CA). Buffer A: 50 mM Tris HCl, 150 mM NaCl, 30 mM imidazole, 2 mM MgCl2, 0.1 mM TCEP, pH 8.0 (4 °C). Buffer B: 50 mM Tris HCl, 150 mM NaCl, 300 mM imidazole, 2 mM MgCl2, 0.1 mM TCEP, pH 8.0 (4 °C). The target protein in the soluble lysate fraction was batch bound to 4 mL of Ni-NTA resin at 4 °C for 2 h. The resin was collected in a 1.0 cm diameter Econo-column, was washed with 20 CV of buffer A, and the protein of interest was eluted in buffer B. Fractions
(1 mL) were collected. The final destination buffer was 100 mM potassium phosphate, 1 mM DTT, 5 mM MgCl2, 20% glycerol at pH 7.2.

**Compound Dilution:**

All compounds were diluted to a final concentration of 1.5% DMSO from 10 mM stocks in two steps. Stock (7.5 μL) was diluted serially into 15 μL of DMSO. Then 4.5 μL of the previous was diluted into 55.5 μL of COMT assay buffer (50 mM Tris, 5–10 mM MgCl2, 2.5 mM DTT, pH 6.9). Then 1 μL of that was then deposited into a Corning low volume 384-well white flat-bottom polystyrene NBS microplate in triplicate.

For 0 and 100% inhibition controls, DMSO and tolcapone (synthesized in house) were added to each assay plate. DMSO was added to 12 wells on the plate in a final concentration of 1.5%, and tolcapone was added to 12 wells on the plate with a final concentration of 10 μM.

Tolcapone was diluted to 666.6 μM and then diluted in two steps according to the procedure of the compounds above. DMSO (MilliporeSigma, St. Louis, MO) was also diluted according to the same scheme.

**Enzyme Reaction:**

COMT activity was measured using the MTase Glo methyltransferase assay (Promega, Madison, WI) according to manufacturer’s instructions. Assays were carried out in Corning low volume 384-well white flat-bottom polystyrene NBS microplates with a final volume of 5 μL containing approximately 7 ng of human MB-COMT as estimated by the Bradford Lowry method from the membrane homogenate, 4 ng of rat MB-COMT as estimated by the Bradford Lowry method from the membrane homogenate, 1 ng of Human S-COMT, or 1 ng of Rat S-COMT respectively. All reactions contained 20 μM high purity S-adenosyl methionine (SAM, CisBio, Bedford, MA) in COMT assay buffer (50 mM Tris, 5–10 mM MgCl2, 2.5 mM DTT, pH 6.9). For MB-COMT,
the catechol substrate was 7 μM norepinephrine (MilliporeSigma, St. Louis, MO) and for S-COMT the substrate was 10 μM 7,8-dihydroxy-4-methylcoumarin(32) (MilliporeSigma, St. Louis, MO).

Reactions were performed in a 37 °C incubator for 1 h. The plate was removed from the incubator and allowed to cool to room temperature for 15 min. MTase reagent A (Promega, Madison, WI) was first diluted 1:5 into RO water, and 1 μL was then added to the well. The plate was spun down, shaken, and allowed to incubate for 30 min at room temperature avoiding light. Then 5 μL of MTase reagent B (Promega, Madison, WI) were added to all of the wells. The plate was spun down, shaken, and allowed to incubate for 30 min at room temperature avoiding light. Luminescence was detected with a Tecan Infinite M100 Pro plate reader.

**Standard Curve:***

A standard curve was run on every plate. The amount of S-adenosyl homocysteine (SAH, CisBio, Bedford, MA) produced was determined using a standard curve and a linear back-calculation method. The standard curve comprised of varying concentrations of SAH from 500 nM down to 0 nM while maintaining a final SAM/SAH concentration of 50 μM. To correct for background levels present in the enzymatic lysate (MB-COMT), enzyme at assay concentration was added to the standard curve as well.

**Determination of Inhibition:**

Percentage inhibition was calculated by using 10 μM tolcapone value as 100% inhibition value and the DMSO control as the 0% inhibition value. The dose response curves were constrained at 0% inhibition while keeping the percentage inhibition of the highest compound concentration floating. IC50 was determined by nonlinear regressions and curve fitting using a four-parameter
fit with a variable slope in the Dotmatics studies program. Potency data presented is an average of three separate experiments in which each data point was run in triplicate.

| Cmpd | Human S-COMT IC\textsubscript{50} (µM) |
|------|--------------------------------------|
| 19   | 61.0                                 |
| 31   | 52.0                                 |
| 40   | 90.5                                 |
| 38   | 12.5                                 |
| 35   | 5.2                                  |

All data are the mean values of at least three independent measurements.