

# Supporting Information

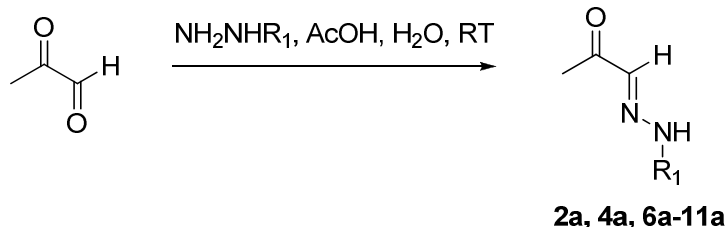
## Maximizing Lipophilic Efficiency: The Use of Free-Wilson Analysis in the Design of Inhibitors of Acetyl-CoA Carboxylase

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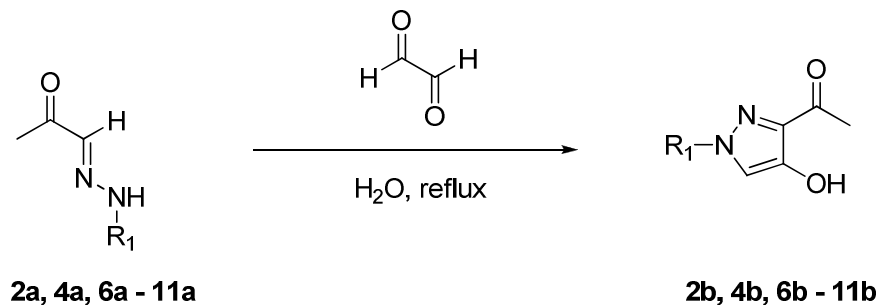
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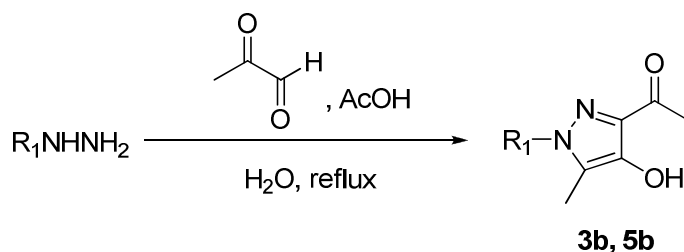
## I. General Procedures and Synthesis of Headpiece Intermediates



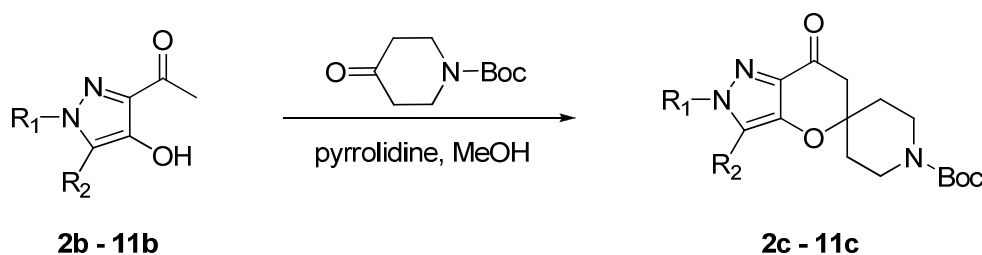
**General Procedure I: Synthesis of hydrazones (2a, 4a, 6a – 11a).** Acetic acid (0.120 mL/1 mmol of pyruvaldehyde) was added slowly to a solution of the appropriate hydrazine or hydrazine salt (1-1.2 eq). Then, pyruvaldehyde (1 eq) was added slowly. The resulting aqueous mixture was then either stirred overnight at room temperature or heated at reflux for several hours. If necessary, the mixture was cooled to rt and extracted with  $\text{CH}_2\text{Cl}_2$  (3x). The combined organic layers were washed with saturated sodium bicarbonate, dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure to afford the hydrazone. The hydrazone could be further purified via medium pressure flash chromatography (silica gel) eluting with a heptane:ethyl acetate gradient. Note: No acetic acid was used in the synthesis of hydrazones **4a**, **8a**, and **12a**. Yields: **2a**  $\text{R}_1 = \text{Me}$  (45%), **4a**  $\text{R}_1 = \text{Et}$  (48%), **6a**  $\text{R}_1 = n\text{-Pr}$  (96%), **7a**  $\text{R}_1 = i\text{-Pr}$  (56%), **8a**  $\text{R}_1 = \text{Bn}$  (100%), **9a**  $\text{R}_1 = c\text{-butyl}$  (40%), **10a**  $\text{R}_1 = c\text{-pentyl}$  (60%), **11a**  $\text{R}_1 = c\text{-hexyl}$  (45%)



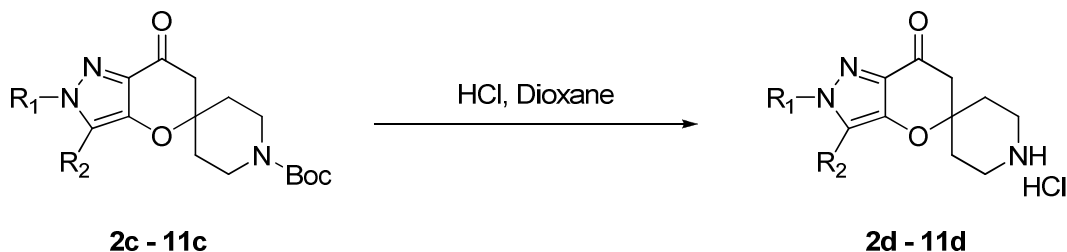
**General Procedure II: Synthesis of pyrazoles 2b, 4b, 6b-11b:** A 40% aqueous solution of glyoxal (1 eq) was added to the hydrazone (1 eq). The mixture was then heated at reflux. After 1 hour, the mixture was cooled to room temperature and extracted with  $\text{CH}_2\text{Cl}_2$  (4x). The combined organic extracts were dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure to the requisite pyrazole. The pyrazole could be further purified via medium pressure flash chromatography (silica gel) eluting with a heptane:ethyl acetate gradient. **2b**  $\text{R}_1 = \text{Me}$  (24%), **4b**  $\text{R}_1 = \text{Et}$  (87%), **6b**  $\text{R}_1 = n\text{-Pr}$  (73%), **7b**  $\text{R}_1 = i\text{-Pr}$  (82%), **8b**  $\text{R}_1 = \text{Bn}$  (33%), **9b**  $\text{R}_1 = c\text{-butyl}$  (86%), **10b**  $\text{R}_1 = c\text{-pentyl}$  (53%), **11b**  $\text{R}_1 = c\text{-hexyl}$  (ND).



**General Procedure III: Synthesis of 5-methylpyrazoles **3b** and **5b**:** An aqueous solution of methyl glyoxal (pyruvaldehyde) (40%, 6 eq) was added to a solution of the appropriate hydrazine (1 eq) and acetic acid (1.5 eq). The resulting mixture was heated at reflux for 3 hours. The reaction mixture was cooled to room temperature and extracted with EtOAc (3x). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel) eluting with a gradient of heptane:ethyl acetate to give the 5-methylpyrazole. **3b**  $R_1 = \text{Me}$  (100%), **5b**  $R_1 = \text{Et}$  (56%).



**General Procedure IV: Synthesis of 2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one cores (**2c** - **11c**):** Pyrrolidine (1 eq) was added to a solution of the pyrazole (1 eq) in MeOH. The solution was stirred for 2 h at room temperature. 1-(N-Boc)-4-piperidone (1 eq) was then added to the solution, and the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated under reduced pressure. The crude product was then purified via flash chromatography (silica gel) eluting with a gradient of ethyl acetate:hexanes (30:70 to 50:50) to the desired product. **2c**  $R_1 = \text{Me}$ ,  $R_2 = \text{H}$  (45%), **3c**  $R_1 = \text{Me}$ ,  $R_2 = \text{Me}$  (36%), **4c**  $R_1 = \text{Et}$ ,  $R_2 = \text{H}$  (51%), **5c**  $R_1 = \text{Et}$ ,  $R_2 = \text{Me}$  (59%), **6c**  $R_1 = n\text{-Pr}$ ,  $R_2 = \text{H}$  (26%), **7c**  $R_1 = i\text{-Pr}$ ,  $R_2 = \text{H}$  (37%), **8c**  $R_1 = \text{Bn}$ ,  $R_2 = \text{H}$  (40%), **9c**  $R_1 = c\text{-butyl}$ ,  $R_2 = \text{H}$  (27%), **10c**  $R_1 = c\text{-pentyl}$ ,  $R_2 = \text{H}$  (25%), **11c**  $R_1 = c\text{-hexyl}$ ,  $R_2 = \text{H}$  (6%).



**General Procedure V: BOC-deprotection (2d – 11d):** To a solution of a BOC-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one ( 1eq) in dioxane was added 4 M HCl in dioxane (10 eq). The mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the resulting solid was triturated with 2-methyltetrahydrofuran or diethyl ether and a small amount of ethanol. The solids were collected by filtration and air dried to afford the piperidinium chloride. **2d** R<sub>1</sub> = Me, R<sub>2</sub> = H (75%), **3d** R<sub>1</sub> = Me, R<sub>2</sub> = Me (88%), **4d** R<sub>1</sub> = Et, R<sub>2</sub> = H (76%), **5d** R<sub>1</sub> = Et, R<sub>2</sub> = Me (92%), **6d** R<sub>1</sub> = n-Pr, R<sub>2</sub> = H (86%), **7d** R<sub>1</sub> = *i*-Pr, R<sub>2</sub> = H (68%), **8d** R<sub>1</sub> = Bn, R<sub>2</sub> = H (100%), **9d** R<sub>1</sub> = *c*-butyl, R<sub>2</sub> = H (57%), **10d** R<sub>1</sub> = *c*-pentyl, R<sub>2</sub> = H (64%), **11d** R<sub>1</sub> = *c*-hexyl, R<sub>2</sub> = H (80%)

**Amidation Method A:** To a flask was added the appropriate amine or amine hydrochloride (1equivalent), DMF, DMSO or CH<sub>2</sub>Cl<sub>2</sub> (about 0. 1 M), carboxylic acid, N,N-diisopropylethylamine (DIEA) (4-6 equivalents) or triethylamine (TEA) (4-6 equivalents) and 2- (7-aza-1*H*-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate (HATU) (1-1.3 equivalents) or 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (EDCI) (1 equivalent) with or without N-Hydroxy-benzotriazole (HOBT) (1 equivalent). The mixture was stirred at room temperature until the reaction was complete as determined by LC/MS. The mixture was diluted with ethyl acetate or CH<sub>2</sub>Cl<sub>2</sub> and washed with either saturated aqueous NaHCO<sub>3</sub> (2x) aqueous NaOH (0.5 M solution) and then saturated aqueous NaCl. The organic extract was dried over MgSO<sub>4</sub>, filtered and concentrated. The crude material was purified by liquid chromatography to afford product.

**Amidation Method B:** A mixture of carboxylic acid (1 equivalent), 2-chloro-4, 6-dimethoxy-1, 3, 5-triazine (1 equivalent) and N-methylmorpholine (NMM) (1 equivalent) in DMF and/or THF was stirred at room temperature for 1 hour before addition of the amine (1 equivalent) as well as additional NMM (1 equivalent). The resultant mixture was stirred overnight at room temperature. The solution was diluted with EtOAc and washed with saturated aqueous NH<sub>4</sub>Cl. The layers were separated and the aqueous layers were washed with EtOAc. The combined organic extract was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude material was purified by chromatography.

### General Methods for HPLC Analysis:

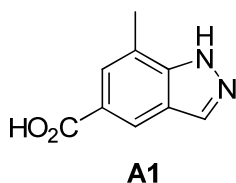
**Method LC-1:** Waters ACQUITY Ultra Performance LC BEH C18 Column, 2.1x30 mm, 1.7 um, 0.05% TFA 95/5 to 5/95 H<sub>2</sub>O/ACN. Flow rate: 1.3 mL/min, run time: 1.1 min.

**Method LC-2:** Waters XTerra C18 4.6x50 mm, 3.5 um column. Solvents A and B are H<sub>2</sub>O w/0.1% TFA and ACN w/0.1% TFA, respectively. 9 min total method time with 5%B to 95% B over 5.83 min. Mass spectral data were acquired from 180-850 amu in electrospray positive mode. Flow rate: 2.0 mL/min.

## II. Synthesis of Analogs 1-12

**6,7-Dimethyl-1'-(7-methyl-1*H*-indazole-5-carbonyl)-spiro[chroman-2,4'-piperidin]-4-one (1).** Compound **1** was prepared according to the general procedures described in reference 15: Corbett, J. W.; et al *Bioorg. Med. Chem. Lett.* **2010**, 20 (7), 2383-2388. <sup>1</sup>HNMR (400 MHz, CDCl<sub>3</sub>) δ 2.20 (s, 3H), 2.26 (s, 3H), 2.66 (m, 3H), 6.80 (s, 1H), 7.33 (s, 1H), 7.59 (s, 1H), 7.71 (s, 1H), 8.24 (br s, 1H).

**2'-Methyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (2).**



**Step 1: Synthesis of 7-methyl-1*H*-indazole-5-carboxylic acid (A1).** To a solution of 5-bromo-7-methylindazole, (purchased from PharmaLab, Morrisville, PA) (2.00 g, 9.47 mmol) in anhydrous THF (50 mL) was added NaH (570 mg, 14.25 mmol; 60% suspension in mineral oil) at room temperature. After 20 minutes the mixture was cooled to -78 °C and *sec*-butyllithium (1.4 M in cyclohexane, 17 mL; 23.8 mmol) was added dropwise and the resulting mixture was stirred for 4 h. Dry CO<sub>2</sub> was then bubbled through the reaction mixture for 1 h while allowing warming to room temperature. The mixture was stirred at room temperature overnight, 1 N HCl was added and the solution extracted with EtOAc. The organic layer was washed with saturated aqueous NaCl, dried (anhydrous MgSO<sub>4</sub>), then filtered and concentrated. The residue was re-dissolved in MeOH, filtered, then concentrated to provide the product (**A1**) as a brown solid (1.445 g, 86.6%). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.46 (s, 3H), 7.65 (s, 1H), 8.17 (s, 1H), 8.23 (s, 1H). LC/MS ES+ 177 (MH+).

**Step 2: 2'-Methyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (2).** Compound **2** was prepared from **2d** and **A1** according to amidation method A (21% yield): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.65 - 1.74 (m, 2 H), 2.50 (s, 3 H), 2.71 (s, 2, 1.029 H), 3.83 (s, 3 H), 7.14 (s, 1 H), 7.56 (s, 1 H), 7.62 (s, 1 H), 8.09 (d, J=1.61 Hz, 1 H) 13.28 (s, 1 H); MS ES+ *m/z* 380 (MH+); HPLC (Method LC-1) Retention time = 0.29 min.

**2',3'-Dimethyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (3).** Compound **3** was prepared from **3d** and **A1** according to amidation

method A (35% yield): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.2 (s, 3 H), 2.56 (s, 3 H), 2.67 (s, 2 H), 3.82 (s, 3 H), 7.23 (s, 1 H), 7.66 (s, 1 H), 8.11 (s, 1 H); MS ES+ *m/z* 394 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.32 min.

**2'-Ethyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (4).** Compound **4** was prepared from **4d** and **A1** according to amidation method B (57% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.44 (t, *J* = 7.27 Hz, 3 H), 2.53-2.63 (m, 4 H), 2.75 (s, 3 H), 4.05-4.25 (m, 4 H), 7.22 (s, 1 H), 7.46 (s, 1 H), 7.71 (s, 1 H), 8.10 (s, 1 H); MS ES+ *m/z* 394 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.32 min

**2'-Ethyl-3'-methyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (5).** Compound **5** was prepared from **5d** and **A1** according to amidation method B (54% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.38 (t, *J* = 7.27 Hz, 3 H), 1.77 (br. s., 2 H), 2.27 (s, 3 H), 2.58 (s, 3 H), 2.73 (s, 2 H), 4.14 (q, *J* = 7.27 Hz, 2 H), 7.23 (s, 1 H), 7.71 (s, 1 H), 8.10 (s, 1 H); MS ES+ *m/z* 408 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.37 min.

**1-(7-Methyl-1*H*-indazole-5-carbonyl)-2'-propyl-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (6).** Compound **6** was prepared from **6d** and **A1** according to amidation method B (64% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 0.89 (t, *J* = 7.27 Hz, 3 H), 1.62-1.97 (m, 5 H), 2.58 (s, 3 H), 2.66-2.87 (m, 2 H), 4.09 (t, *J* = 7.27 Hz, 2 H), 7.22 (s, 1 H), 7.45 (s, 1 H), 7.71 (s, 1 H), 8.10 (s, 1 H); MS ES+ *m/z* 408 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.36 min.

**2'-Isopropyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (7).** Compound **7** was prepared from **7d** and **A1** according to amidation method B (43% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.22 (t, *J* = 7.27 Hz, 1 H), 1.43 (d, *J* = 6.65 Hz, 2 H), 1.47 (d, *J* = 7.06 Hz, 3 H), 1.99 (s, 1 H), 2.27 (s, 1 H), 2.58 (s, 4H), 2.71-2.79 (m, 2H), 4.46-4.55 (m, 1 H), 4.56-4.63 (m, 1H), 7.22 (s, 1 H), 7.49 (s, 1 H), 7.71 (s, 1H), 8.10 (s, 1 H); MS ES+ *m/z* 408 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.36 min.

**2'-Benzyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (8).** Compound **8** was prepared from **8d** and **A1** according to amidation method A (18% yield): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.65 (s, 3 H), 2.70 (s, 2 H), 7.03 (s, 1 H), 7.26-7.37 (m, 5 H), 7.69 (s, 1H), 8.22 (s, 1 H); MS ES+ *m/z* 456 (MH<sup>+</sup>); HPLC (Method LC-1) Retention time = 0.40 minutes.

**2'-Cyclobutyl-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (9).** Compound **9** was prepared from **9d** and **A1** according to amidation method B (68% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.68-1.96 (complex, 5 H), 1.99-2.30 (complex, 3 H), 2.40-2.50 (complex, 2 H), 2.51-2.57 (complex, 2 H), 2.60 (s, 3 H), 2.78 (s, 2 H),

4.77-4.87 (m, 1H), 7.24 (s, 1H), 7.53 (s, 1H), 7.73 (s, 1 H), 8.12 (s, 1 H); MS ES+  $m/z$  420 (MH+); HPLC (method LC-1) retention time = 0.39 min.

**2'-Cyclopentyl-1-(7-methyl-1H-indazole-5-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2-c]pyrazol]-7'(6'H)-one (10).** Compound **10** was prepared from **10d** and **A1** according to amidation method B (65% yield):  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.66 - 2.29 (complex, 14 H), 2.58 (s, 3 H) 2.77 (s, 2 H), 4.63- 4.79 (m, 1 H), 7.24 (s, 1 H), 7.51 (s, 1 H), 7.73 (s, 1 H), 8.12 (s, 1 H); MS ES+  $m/z$  434 (MH+); HPLC (Method LC-1) Retention time = 0.41 min.

**2'-Cyclohexyl-1-(7-methyl-1H-indazole-5-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2-c]pyrazol]-7'(6'H)-one (11).** Compound **11** was prepared from **11d** and **A1** according to amidation method B (47% yield):  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.58 (s, 9 H), 1.77 (br. s., 2 H), 2.12 (br. s., 2 H) 2.60 (s, 3 H), 2.77 (s, 2 H), 7.24 (s, 1 H), 7.61 (s, 1 H), 7.73 (s, 1 H), 8.12 (s, 1 H); MS ES+  $m/z$  448 (MH+); HPLC (Method LC-1) Retention time = 0.44 min.

### Synthesis of 12a-12d:

**(E,Z)-1-(2-tert-Butyl)hydrazono)propan-2-one (12a).** A solution of pyruvaldehyde (43.4 g, 0.241 mol) in water (230 mL) was quickly added to a solution of *t*-butylhydrazine hydrochloride in water (978 mL) at ambient temperature. This solution was then stirred at room temperature for 1.5 h. The reaction mixture was then extracted with dichloromethane (5x) followed by  $\text{CHCl}_3$  (5x). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure with no heating (volatile product). The resulting oil was then purified via medium pressure chromatography ( $\text{SiO}_2$ ) using a hexanes:EtOAc gradient (100:0 to 70:30) to deliver 26.5 g (98%) of the **12a** as a yellow oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.31 (s, 9 H), 2.31 (s, 3 H), 6.37 (br. s., 1 H), 6.95 (s, 1 H).

**1-(1-(tert-Butyl)-4-hydroxy-1H-pyrazol-3-yl)ethanone (12b).** A 40% aqueous solution of glyoxal (62.5 mL, 0.545 mol) was added to a solution of **12a** (25.81 g, 0.182 mol) in water (303 mL). The reaction mixture was then heated at reflux for 5 h. After cooling to room temperature, the aqueous mixture was extracted with EtOAc (4x). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ), filtered, and concentrated under reduced pressure. The crude product was then purified via medium pressure chromatography ( $\text{SiO}_2$ ) eluting with a hexanes:ethyl acetate gradient (100:0 to 85:15) to afford 33.07 g (57%) of the **12b** as a light amber oil.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  1.58 (s, 9 H), 2.55 (s, 3 H), 7.17 (s, 1 H), 8.03 (s, 1 H); MS ES+  $m/z$  183.3 (M+H).

**tert-Butyl-2'-(tert-butyl)-7'-oxo-6',7'-dihydro-2'H-spiro[piperidine-4,5'-pyrano[3,2,-c]pyrazole]-1-carboxylate (12c).** A solution of **12b** (18.69 g, 0.103 mol), 1-(BOC)-4-piperidine (20.4 g, 0.103 mol), and pyrrolidine (2.19 g, 0.0308 mol) in MeOH (186 mL) was heated at reflux for 18 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was then triturated with hexanes:EtOAc (98:2, 500 mL). The suspension was then filtered, and the resulting solid was washed with hexanes and dried *in vacuo*

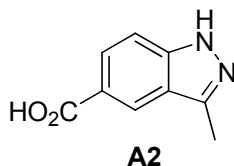
to afford 22.08 g of desired product. The filtrate was then concentrated under reduced pressure to afford a yellow solid was triturated with hexanes:EtOAc (98:2), filtered, and dried in vacuo to deliver another 5.08 g of desired product. This second filtrate was then concentrated to give a solid which was purified via medium pressure chromatography (SiO<sub>2</sub>) eluting with a hexanes:EtOAc gradient (100:0 to 50:50) to afford another 4.28 g of product. The batches were combined to give 31.44 g (84%) of **12c** as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.48 (br. s., 9 H), 1.53-1.69 (c, 2 H) 1.63 (s., 9 H) 2.06 (br. s., 2 H), 2.67 (s, 2 H), 3.17 (br. s., 2 H), 3.87 (br. s., 2 H), 7.23 (s, 1 H); MS ES+ *m/z* 364.5 (M+H).

**2'-(tert-Butyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2,-c]pyrazol]-7'(6'H)-one hydrochloride (12d).** A white suspension of **12c** (15.42 g, 42.42 mmol) in dioxane (60 mL) was treated with 4 M HCl (4 M in dioxane, 42.4 mL). The resulting orange solution was then stirred at ambient temperature for 3 h. TLC indicated starting material, so an additional quantity of 4 M HCl (4 M in dioxane, 21.2 mL) was added. The mixture was stirred for an additional hour. The solvent was then removed under reduced pressure to deliver an orange solid. The solid was then triturated with 2-methyl THF and a trace amount of EtOH for 1 h. The suspension was filtered, and the resulting solid was washed with 2-methyl THF and dried in vacuo to deliver 12.73 g (quantitative) of **12d** as an off-white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.51 (s, 9 H), 1.80-1.95 (complex, 2 H) 2.10 (complex, 2 H), 2.77 (s, 2 H), 2.91 - 3.06 (complex, 2 H), 3.17 (complex, 2 H), 7.83 (s, 1 H), 8.87 (br. s., 1 H) 9.17 (br. s., 1 H). MS ES+ *m/z* 264.4 (M+H).

**2'-(tert-Butyl)-1-(7-methyl-1*H*-indazole-5-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2,-c]pyrazol]-7'(6'H)-one (12).** Compound **12** was prepared from **12d** and **A1** according to amidation method B (70% yield): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.57 (s, 9 H), 2.58 (s, 3 H), 2.75 (s, 2 H), 7.22 (s, 1 H), 7.59 (s, 1 H), 7.71 (s, 1 H), 8.10 (s, 1 H); MS ES+ *m/z* 422 (MH+); HPLC Method LC-1 Retention time = 0.39 min.

### III. Synthesis of carboxylic acid tailpiece intermediates.

#### Synthesis of 3-methyl-1*H*-indazole-5-carboxylic acid (**A2**):



**Step 1: 5-Bromo-3-methyl-1*H*-indazole.** To a cooled solution of 3-bromobenzaldehyde (42.5 g, 209 mmol) in THF (300 mL) at 0 °C was added MeMgCl (17.2 g, 230 mmol) and the mixture was stirred for 2 h. The reaction mixture was quenched with saturated NH<sub>4</sub>Cl solution (100 mL) and extracted with diethyl ether (2x250 mL). The combined organic layers were washed with

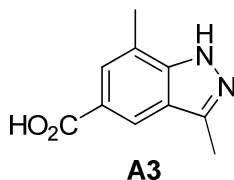


water (2x100 mL), saturated aqueous NaCl (100 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give 17 g (37%) of 1-(5-bromo-2-fluoro-phenyl)-ethanol as an oil. To a solution of 1-(5-bromo-2-fluoro-phenyl)-ethanol (37 g, 168mmol) in CH<sub>2</sub>Cl<sub>2</sub> (400 mL) and pyridinium dichromate (127 g, 337mmol) were added molecular sieves (10 g). The mixture was stirred at room temperature for 20 h. The mixture was filtered through diatomaceous earth, washed with CH<sub>2</sub>Cl<sub>2</sub> (3x100 mL) and concentrated. The crude product was purified by column chromatography (silica gel) eluting with petroleum ether:ethyl acetate (90:10) to afford 23 g (63%) of 1-(5-bromo-2-fluoro-phenyl)-ethanone as pale brown oil. A solution of 1-(5-bromo-2-fluoro-phenyl)-ethanone (10 g, 46 mmol) in hydrazine hydrate (80 mL) was heated at 130 °C for 20 h. The reaction mixture was cooled to room temperature, excess hydrazine hydrate was removed and the crude residue was purified by column chromatography (silica gel) eluting with petroleum ether:ethyl acetate (90:10) to obtain 7.09 g (73%) of 5-bromo-3-methyl-1*H*-indazole as a solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.57 (s, 3 H), 7.28 (d, *J* = 9.2 Hz, 1 H), 7.45 (dd, *J* = 9.2, 1.4 Hz, 1 H), 7.82 (s, 1 H), 10.40 (br. s., 1 H); MS APCI *m/z* 211 (M+H), 213.2 (M+H).

**Step 2: 3-Methyl-1*H*-Indazole-5-carboxylic acid methyl ester.** To a solution of 5-bromo-3-methyl-1*H*-indazole (10 g, 47.6 mmol) in MeOH (160 mL), PdCl<sub>2</sub>dppf (5.57 g, 7.62 mmol), NaOAc (11.7 g, 142.85 mmol) and DMF (5 mL) were added and degassed (using N<sub>2</sub> gas 3-times). The mixture was sealed, charged with CO gas (60 psi) and heated at 80 °C for 20 h. The reaction mixture was concentrated to obtain a residue, which was diluted with water (100 mL), acidified with 10% aqueous citric acid and extracted with ethyl acetate (3x200 mL). The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to afford 7.23 g (80%) of 1*H*-indazole-5-carboxylic acid methyl ester as a solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.63 (s, 3 H), 3.96 (s, 3 H), 7.43 (d, *J* = 8.8 Hz, 1 H), 8.07 (d, *J* = 8.8 Hz, 1 H), 8.48 (s, 1 H), 10.0 (br. s., 1 H); MS APCI *m/z* 191.3 (M+H).

**Step 3: 3-Methyl-1*H*-indazole-5-carboxylic acid (A2).** A solution of 1*H*-indazole-5-carboxylic acid methyl ester (8 g, 42.1 mmol), LiOH (5.05 g, 211 mmol), in methanol (100 mL) and water (35 mL) was stirred at room temperature for 20 h. At the end of this time, the reaction mixture was concentrated, the aqueous residue was acidified (pH~6) using 10% aqueous citric acid (150 mL) and filtered the solid to obtain the crude product. The solid was washed with water (3 x 30mL) and dried in vacuo to give 7 g (95%) of 3-methyl-1*H*-indazole-5-carboxylic acid (**A2**) as a pale brown solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 2.61 (s, 3H), 7.52 (d, *J* = 8.8 Hz, 1 H), 8.05 (dd, *J* = 8.8, 1.5 Hz, 1 H), 8.49 (s, 1H); MS APCI *m/z* 177.3 (M+H).

#### Synthesis of 3,7-Dimethyl-1*H*-indazole-5-carboxylic acid (**A3**):



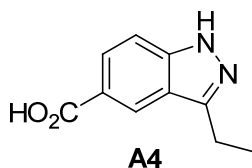
**Step 1: 4-Bromo-2-ethyl-6-methylbenzenamine.** To a solution of 2-ethyl-6-methylaniline (2.03 g, 15 mmol) in DMF (50 mL) at 0 °C was added *N*-bromosuccinimide (2.66 g, 14.9 mmol). The

mixture was stirred at room temperature for 10 min before addition to saturated aqueous NaCl. The mixture was then extracted with EtOAc. The organic phase was washed with sat. aqueous NaCl (2x), concentrated and the crude material was purified by chromatography (silica gel) eluting with heptane:ethyl acetate (85:15) to provide 3.21 g (100%) of 4-bromo-2-ethyl-6-methylbenzenamine as a red brown liquid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.25 (d, *J* = 7.5 Hz, 3 H) 2.16 (s, 3 H) 2.50 (q, *J* = 7.5 Hz, 2 H) 3.60 (br. s., 2 H) 7.08 (s, 2 H).

**Step 2: 5-Bromo-3,7-dimethyl-1H-indazole.** A solution of 4-bromo-2-ethyl-6-methylbenzenamine (3.21 g, 15 mmol) in acetic acid (50 mL) was stirred for 3 h before addition of a 2 M solution of sodium nitrite (11 mL, 22.5 mmol). The resulting mixture was stirred overnight at room temperature. The solution was concentrated and the solid was dissolved in EtOAc and washed with saturated aqueous NaCl (3x). The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The crude material was purified by chromatography (silica gel) eluting with a gradient of heptane:ethyl acetate (85:15 to 70:30) to provide 1.1 g (33%) of 5-bromo-7-ethyl-1H-indazole and 0.84 g (25%) of 5-bromo-3,7-dimethyl-1H-indazole. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 2.51 (s, 3 H), 2.56 (s, 3 H), 7.26 (d, *J* = 1.3 Hz, 1 H), 7.67 (d, *J* = 1.3 Hz, 1 H); MS ESI+ *m/z* 225.1 (M+H), 227.1 (M+H).

**Step 3: 3,7-Dimethyl-1H-indazole-5-carboxylic acid (A3).** To a reaction vessel containing 5-bromo-3,7-dimethyl-1H-indazole (285 mg, 1.27 mmol) in dioxane (1.3 mL) was added hexacarbonylmolybdenum (264 mg, 1.0 mmol), Herrmann's catalyst (93 mg, 0.1 mmol) and an aqueous solution of Na<sub>2</sub>CO<sub>3</sub> in water (636 mg in 2 mL of water). The suspension was heated in a microwave at 165 °C for 15 minutes (high absorption). The vial was vented, acidified with 1 N HCl (to pH 2). The reaction mixture was filtered through diatomaceous earth, washed with EtOAc and the organic layer was washed with saturated aqueous NaCl (3x). The organic extract was concentrated to yield 65 mg (17%) of 3,7-dimethyl-1H-indazole-5-carboxylic acid (**A3**) as a pink solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.50 (s, 3 H), 2.55 (s, 3 H), 3.90 (s, 3 H), 7.75 (s, 1 H), 8.25 (s, 1 H); MS ESI+ *m/z* 177.1 (M+H).

#### Synthesis of 3-ethyl-1H-indazole-5-carboxylic acid (A4):

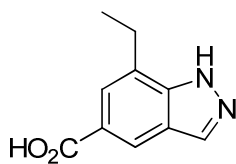


**Step 1: 5-Bromo-3-ethyl-1H-indazole.** To a solution of 5-bromo-2-fluorobenzaldehyde (10 g, 49 mmol) in diethyl ether (20 mL) at 15 °C was added dropwise at 15 °C EtMgBr (7.22 g, 54.2 mmol). The mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with cold water and NH<sub>4</sub>Cl solution. It was extracted with diethyl ether, and the ether layer was washed with saturated aqueous NaCl, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford 12.1 g (98%) of 1-(5-bromo-2-fluorophenyl)propan-1-ol as a syrup. To 1-(5-bromo-2-fluorophenyl)propan-1-ol (12.1 g, 64.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added pyridinium dichromate (73.2 g, 194 mmol) and powdered molecular sieves. The mixture was stirred at room temperature for 16 h. The reaction mixture was filtered through a pad of

diatomaceous earth and washed with diethyl ether. The combined ether and CH<sub>2</sub>Cl<sub>2</sub> layers were collected and concentrated under vacuum to afford 12.6 g (98%) of 1-(5-bromo-2-fluorophenyl)propan-1-one as a syrup. To 1-(5-bromo-2-fluorophenyl)propan-1-one (10 g, 43 mmol), anhydrous hydrazine (53 mL) was added and heated at 70 °C for 2 d. Excess hydrazine was distilled under reduced pressure from the reaction mixture. To this mixture, xylene (60 mL) was added, and the mixture was heated to 145-150 °C for 3 d. Xylene was removed under reduced pressure to give a crude product, which was purified by column chromatography (silica gel) eluting with petroleum ether:ethyl acetate (92:8) to afford 4 g (40%) of 5-bromo-3-ethyl-1H-indazole as a solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.41 (t, *J* = 8.3 Hz, 3 H), 3.0 (q, *J* = 8.3 Hz, 2 H), 7.32 (d, *J* = 8.8 Hz, 1 H), 7.45 (dd, *J* = 8.8, 1.5 Hz, 1 H), 7.86 (s, 1 H), 9.80 (br, 1 H); MS APCI 225.3 (M+H), 227.3 (M+H).

**Step 2: 3-Ethyl-1H-indazole-5-carboxylic acid (A4).** To a -78 °C solution of 5-bromo-3-ethyl-1H-indazole (5 g, 22 mmol) in diethyl ether (150 mL) was added *t*-BuLi (4.84 g, 75.6 mmol). The mixture was stirred at -78 °C for 1 hour. Dry CO<sub>2</sub> gas was passed through the reaction mixture at -78 °C for 1 h and the temperature was slowly raised to room temperature over 2 h. The reaction mixture was stirred at room temperature for 1 h and quenched with saturated NH<sub>4</sub>Cl solution. The ether layer was separated and concentrated to recover unreacted starting material. The pH of the aqueous layer was made acidic with 2 N HCl, which was filtered to afford 2 g (47%) of 3-Ethyl-1H-indazole-5-carboxylic acid (A4) as a pale brown solid. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.33 (t, *J* = 7.8 Hz, 3H), 3.00 (q, *J* = 7.8 Hz, 2 H), 7.52 (d, *J* = 8.3 Hz, 1 H), 7.90 (d, *J* = 8.3 Hz, 1 H), 8.39 (s, 1 H), 12.9 (br, 2 H); MS APCI 191.3 (M+H).

#### Synthesis of 7-Ethyl-1H-indazole-5-carboxylic acid (A5):



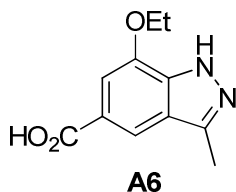
**A5**

**Step 1: 5-Bromo-3,7-dimethyl-1H-indazole.** A solution of 4-bromo-2-ethyl-6-methylbenzenamine (3.21 g, 15 mmol) in acetic acid (50 mL) was stirred for 3 h before addition of a 2 M solution of sodium nitrite (11 mL, 22.5 mmol). The resulting mixture was stirred overnight at room temperature. The solution was concentrated and the solid was dissolved in EtOAc and washed with saturated aqueous NaCl (3x). The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated, the crude material was purified by chromatography (silica gel) eluting with a gradient of heptane:ethyl acetate (85:15 to 70:30) to provide 1.1 g (33%) of 5-bromo-7-ethyl-1H-indazole and 0.84 g (25%) of 5-bromo-3,7-dimethyl-1H-indazole. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.40 (t, *J* = 7.5 Hz, 3 H), 2.92 (q, *J* = 7.5 Hz, 2 H), 7.29 - 7.32 (m, 1 H), 7.76 (d, *J* = 1.7 Hz, 1 H), 8.03 (s, 1 H); MS ESI+ *m/z* 225 (M+H), 227 (M+H).

**Step 2: 7-Ethyl-1H-indazole-5-carboxylic acid (A5).** To a solution of 5-bromo-7-ethyl-1H-indazole (225 mg, 1.00 mmol) in dioxane (1.5 mL), hexacarbonylmolybdenum (132 mg, 0.50 mmol), Herrmann's catalyst (trans-Bis(acetato)bis[o-(di-*o*-tolylphosphino)benzyl]dipalladium) (46.9 mg, 0.05 mmol) and a solution of sodium carbonate (318 mg, 3.00 mmol) in water (2 mL).

The suspension was sealed and irradiated in a microwave at 165 °C for 15 min (high absorption setting). The vial was vented, filtered through diatomaceous earth, washed with EtOAc and concentrated to afford 140 mg (74%) of 7-ethyl-1*H*-indazole-5-carboxylic acid (**A5**). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.30 (t, *J* = 7.6 Hz, 3 H), 2.94 (q, *J* = 7.6 Hz, 2 H), 7.71 (s, 1 H), 8.23 (s, 1 H), 8.29 (s, 1 H), 12.64 (br.s., 1H), 13.47 (br.s, 1 H); MS ESI+ *m/z* 191.2 (M+H).

#### Synthesis of 7-Ethoxy-3-methyl-1*H*-indazole-5-carboxylic acid (**A6**):



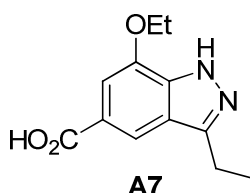
**Step 1: ethyl 3-methyl-7-hydroxy-1*H*-indazole-5-carboxylate** A solution of 1-acetyl-7-(acetyloxy)-3-methyl-1*H*-indazole-5-carboxylate (5.5g, 18.09 mmol, prepared as described in *Synthesis* **1997**, 1140-1142) in ethanol (25mL) and 2N HCl (50mL) was heated to 70 °C and maintained at this temperature for 2 h. The mixture was cooled to rt and concentrated under reduced pressure. The aqueous residue was basified (pH~9) with NH<sub>4</sub>OH, and the mixture was concentrated completely. The resulting solid was suspended in water (25 mL) stirred at rt for 30 min and filtered. The crude product was washed with petroleum ether:acetone (9:1, 2 x 10 mL) and to afford 2 g (50%) of ethyl 3-methyl-7-hydroxy-1*H*-indazole-5-carboxylate as an off white solid. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 1.33 (t, *J* = 7.3 Hz, 3 H), 4.31 (q, *J* = 7.3 Hz, 2 H), 7.26 (s, 1 H), 7.85 (s, 1 H), 10.36 (s, 1 H), 12.96 (br. s., 1 H); MS APCI *m/z* 221.3 (M+H).

**Step 2: Ethyl 7-ethoxy-3-methyl-1*H*-indazole- 5-carboxylate.** To a 0 °C solution of ethyl 3-methyl-7-hydroxy-1*H*-indazole-5-carboxylate (0.84 g, 3.8 mmol) in DMF (25 mL) was added 60 percent oil dispersion of NaH (152 mg, 3.81 mmol). After stirring for 1 h at 0 °C, a solution of iodoethane (595 mg, 0.31 mL, 3.81 mmol) in DMF (3 mL) was added. The mixture was kept at 0 °C for several hours before allowing the mixture to warm to room temperature while stirring overnight. The mixture was diluted with EtOAc, washed with saturated aqueous NaHCO<sub>3</sub>, water, and saturated aqueous NaCl. The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The crude material was purified by medium pressure liquid chromatography (SiO<sub>2</sub>) eluting with a gradient of hexanes:EtOAc (100:0 to 70:30) to provide 516 mg (55%) of ethyl 7-ethoxy-3-methyl-1*H*-indazole- 5-carboxylate. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.44 (t, *J* = 7.1 Hz, 3 H), 1.52 (t, *J* = 6.9 Hz, 3 H), 4.28 (q, *J* = 6.9 Hz, 2 H), 4.42 (q, *J* = 7.1 Hz, 2 H), 7.40 (s, 1 H), 8.07 (s, 1 H), 10.04 (br. s., 1 H); MS ESI+ *m/z* 249.3 (M+H).

**Step 3: 7-Ethoxy-3-methyl-1*H*-indazole-5-carboxylic acid (**A6**).** To a solution of ethyl 7-ethoxy-3-methyl-1*H*-indazole-5-carboxylate (516 mg, 2.08 mmol) in THF (17 mL) was added 1 M LiOH (4.2 mL, 4.2 mmol) and the mixture was heated at reflux overnight. Analysis indicated the presence of small amounts of unreacted starting material; therefore, a small amount of ethanol was added to assist in solubilizing materials. Heating was continued for an additional 2 h before concentrating to dryness. The residue was triturated with 1 N HCl, the solids were isolated by filtration, washed with water and air dried overnight to provide 417 mg (91%) of 7-

ethoxy-3-methyl-1*H*-indazole-5-carboxylic acid (**A6**). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.53 (t, *J* = 7.1 Hz, 3 H), 2.56 (s, 3 H), 4.28 (q, *J*=7.1 Hz, 2 H) 7.39 (s, 1 H) 8.07 (s, 1 H); MS ESI+ *m/z* 221.3 (M+H).

### Synthesis of 7-Ethoxy-3-ethyl-1*H*-indazole-5-carboxylic acid (**A7**):



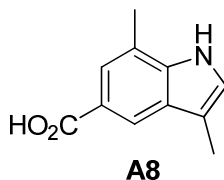
**Step 1: Ethyl 3-ethyl-7-hydroxy-1*H*-indazole-5-carboxylate.** To a solution of 1-acetyl-7-(acetyloxy)-3-ethyl-1*H*-indazole-5-carboxylate (4.0 g, 13 mmol, synthesized analogously to the 3-methyl derivative in *Synthesis* **1997**, 1140-1142) in ethanol (450 mL) was slowly added 60 percent NaH (0.50 g, 12.6 mmol). The mixture was stirred for 1 hour before concentration and removal of the majority of the ethanol. The residue was taken up in EtOAc and washed with saturated aqueous NaHCO<sub>3</sub>. The aqueous phase was back extracted with EtOAc (2x). The combined organic extracts were washed with saturated aqueous NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The solid was triturated with CH<sub>2</sub>Cl<sub>2</sub> to provide 2.9 g (87%) of ethyl 3-ethyl-7-hydroxy-1*H*-indazole-5-carboxylate as an off- white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.30 (t, *J* = 7.48, 3 H), 1.33 (t, *J* = 7.08 Hz, 3 H), 2.92 (q, *J* = 7.48 Hz, 2 H) 4.32 (q, *J* = 7.08 Hz, 2 H) 7.25 (s, 1 H) 7.86 (s, 1 H) 10.41 (s, 1 H) 13.00 (s, 1 H); MS ESI+ *m/z* 235.1 (M+H).

**Step 2: Ethyl 7-ethoxy-3-ethyl-1*H*-indazole-5-carboxylate.** To a 0 °C solution of ethyl 3-ethyl-7-hydroxy-1*H*-indazole-5-carboxylate (932 mg, 3.98 mmol) in DMF (28 ml) was added 60 percent NaH oil dispersion (159 mg, 3.98 mmol). This mixture was stirred at 0 °C for 1 h before the dropwise addition of a solution of iodoethane (0.32 ml, 3.98 mmol) in DMF (3 ml). The mixture was kept at 0 °C for several hours before removal of the cooling bath and allowing the reaction to warm to room temperature while stirring overnight. The reaction was diluted with EtOAc, washed with saturated aqueous NaHCO<sub>3</sub> and saturated aqueous NaCl. The organic extract was then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The crude product was purified by chromatography (silica gel) eluting with a gradient of hexanes:ethyl acetate (100:0 to 70:30) to provide 389 mg (37%) of ethyl 7-ethoxy-3-ethyl-1*H*-indazole-5-carboxylate and 202 mg of ethyl 7-ethoxy-1,3-diethyl-1*H*-indazole-5-carboxylate. <sup>1</sup>H NMR (400 MHz, CHLOROFORM-*d*) δ 1.43 (t, *J* = 7.48 Hz, 3 H), 1.44 (t, *J* = 7.06 Hz, 3 H) 1.52 (t, *J* = 7.06 Hz, 3 H) 3.04 (q, *J* = 7.48 Hz, 2 H) 4.28 (q, *J* = 7.06 Hz, 2 H) 4.42 (q, *J* = 7.06 Hz, 2 H) 7.40 (s, 1 H) 8.10 (s, 1 H) 10.01 (br. s., 1 H); MS ESI+ *m/z* 263.2 (M+H).

**Step 3: 7-Ethoxy-3-ethyl-1*H*-indazole-5-carboxylic acid (**A7**).** To a solution of ethyl 7-ethoxy-3-ethyl-1*H*-indazole-5-carboxylate (389 mg, 1.48 mmol) in THF (13 mL) was added 1 M LiOH (3.0 mL, 3.0 mmol). The mixture was heated at reflux overnight. To the reaction was added trace amounts of ethanol to aid in solubilizing materials. The mixture was heated at reflux for an additional 2 h. The reaction was cooled to room temperature, concentrated, triturated with 1 N

HCl, the filtered solids were then washed with water and air dried to provide 347 mg (97%) of 7-ethoxy-3-ethyl-1*H*-indazole-5-carboxylic acid (**A7**). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.39 (t, *J* = 7.75 Hz, 3 H), 1.53 (t, *J* = 7.06 Hz, 3 H) 3.01 (q, *J* = 7.75 Hz, 2 H) 4.28 (q, *J* = 7.06 Hz, 2 H), 7.39 (s, 1 H), 8.10 (s, 1 H); MS ESI+ *m/z* 235.1 (M+H).

### Synthesis of 3,7-Dimethyl-1*H*-indole-5-carboxylic acid (**A8**):

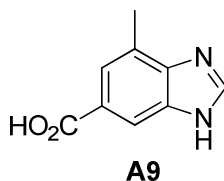


**Step 1: (4-Bromo-2-methylphenyl)hydrazine.** 4-Bromo-2-methylaniline (2.00 g, 10.8 mmol) was dissolved in 12 M HCl (10 mL) and heated to 80 °C for 30 min. The reaction was then cooled to 5 °C and a solution of NaNO<sub>2</sub> (781 mg, 10.8 mmol) in water (4 mL) was added over 10 min. The resulting mixture was stirred at 5 °C for 30 min before addition of SnCl<sub>2</sub> (15.3 g, 80.6 mmol) in 12 M HCl (8 mL) over 10 min. The mixture was stirred at room temperature for 45 min before addition of 50% aqueous NaOH. The resulting precipitate was isolated by filtration and the filtrate was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated to provide 1.08 g (50%) of (4-bromo-2-methylphenyl)hydrazine. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 2.08 (s, 3 H), 6.92 (d, *J* = 8.3 Hz, 1 H), 7.09 (d, *J* = 2.1 Hz, 1 H), 7.18 (dd, *J* = 8.3, 2.1 Hz, 1 H). MS ESI+ *m/z* 201.1 (M+H), 203.1 (M+H).

**Step 2: 5-Bromo-3,7-dimethyl-1*H*-indole.** To a suspension of (4-bromo-2-methylphenyl)hydrazine (1.44 g, 7.16 mmol) in ethanol (10 mL) was added propionaldehyde (0.68 mL, 9.3 mmol). The solution became clear and the mixture was stirred at room temperature for 45 min. The solvents were removed and anhydrous ZnCl<sub>2</sub> (1.07 g, 7.88 mmol) was added and the mixture was heated at 170 °C for 30 min. The mixture was cooled to room temperature, diluted with 10% HCl, extracted with CH<sub>2</sub>Cl<sub>2</sub> and the organic extract was dried (MgSO<sub>4</sub>). After filtration and concentration, the residue was purified by medium pressure liquid chromatography eluting with a heptane:ethyl acetate gradient (100:0 to 90:10) to provide 317 mg (20%) of 5-bromo-3,7-dimethyl-1*H*-indole. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 2.27 (d, *J* = 1.17 Hz, 3 H), 2.44 (s, 3 H), 6.96 (m, 1 H), 7.09 (m, 1 H), 7.55 (m, 1 H), 7.83 (br s, 1 H).

**Step 3: 3,7-Dimethyl-1*H*-indole-5-carboxylic acid (**A8**).** A microwave tube was charged with 5-bromo-3,7-dimethyl-1*H*-indole (317 mg, 1.42 mmol), dioxane (3 mL), Hermann's catalyst (42.1 mg, 0.07 mmol) and a solution of sodium carbonate (450 mg, 4.24 mmol) in water (6 mL). The mixture was stirred for 20 sec, heated at 165 °C for 15 minutes in a microwave reactor (very high absorption). The reaction was vented before handling. The mixture was then filtered through diatomaceous earth and washed with EtOAc. The filtrate was concentrated and the residue was dissolved in water. The solution was acidified to pH 3 and the solid was collected to afford 250 mg (93%) of 3,7-dimethyl-1*H*-indole-5-carboxylic acid (**A8**). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 2.25 (d, *J* = 1.2 Hz, 3 H) 2.44 (s, 3 H), 7.17 (m, 1 H) 7.47 (m, 1 H) 7.98 (s, 1 H) 11.05 (s, 1 H); MS ESI- *m/z* 188 (M-H).

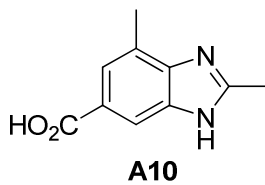
### Synthesis of 4-Methyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (A9):



**Step 1: 6-Bromo-4-methyl-1*H*-benzimidazole.** To a solution of 5-bromo-3-methylbenzene-1,2-diamine (2.02 g, 10 mmol) was added water (10 mL) followed by formic acid (1.16 mL, 30 mmol). The mixture was stirred at 100 °C for 6 h before cooling to room temperature. A solid precipitated from the solution and was allowed to sit at room temperature for 2 d. To this mixture was then added 1 N KOH (35 mL) and the solids were isolated by vacuum filtration. The solids were air dried and recrystallized from CHCl<sub>3</sub> (70 mL) to provide 820 mg (39%) of 6-bromo-4-methyl-1*H*-benzimidazole. Additional batches of product were obtained from the CHCl<sub>3</sub> mother liquors to deliver 1.01 g (48%). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 2.57 (s, 3 H) 7.22 (s, 1 H) 7.60 (s, 1 H) 8.18 (s, 1 H); MS ESI+ *m/z* 211 (M+H), 213 (M+H).

**Step 2: 4-Methyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (A9).** To a solution of 6-bromo-4-dimethyl-1*H*-benzimidazole (100 mg, 0.47 mmol) in degassed dioxane (2 mL) was added ((trans-bis(acetato)bis[o-(di-*o*-tolylphosphino)benzyl]-dipalladium(II))) (16 mg, 0.03 mmol), molybdenum hexacarbonyl (64 mg, 0.24 mmol) and a solution of Na<sub>2</sub>CO<sub>3</sub> (150 mg, 1.42 mmol) in degassed water (2.4 mL). The mixture was stirred for 20 sec and then heated at 165 °C for 15 min in a microwave reactor with absorption set at very high for 20 min. The reaction vessel was vented and filtered through a SPE cartridge. The aqueous solution was extracted with EtOAc (3x). The organic extracts were combined and concentrated. The residue was dissolved in water and acidified to pH 3 and stored in a refrigerator overnight. To the aqueous material was added water (3 mL) and pH 3. A solid precipitated from solution and was isolated by filtration. The solids were washed with water and air dried to provide 44 mg (53%) of 4-methyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (A9). Upon sitting, solids precipitated from the mother liquors to afford an additional 34 mg (41%). The overall yield was 78 mg (94%). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 2.55 (s, 3 H), 7.56 - 7.67 (m, 1 H), 8.02 (s, 1 H), 8.36 (s, 1 H), 12.63 (s, 1 H), 12.70 (s, 1 H), 12.88 (br s, 1 H); MS ESI+ *m/z* 177.1 (M+H).

### Synthesis of 2,4-Dimethyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (A10):



**Step 1: 6-Bromo-2,4-dimethyl-1H-benzimidazole.** To a solution of 5-bromo-3-methylbenzene-1,2-diamine (201 mg, 1.00 mmol) in EtOH (15 mL) was added 5 N HCl (4 mL). The mixture was heated at reflux before addition of 2,4-pentanedione (200 mg, 0.21 mL, 2.0 mmol). Heating was continued for 45 min before analysis indicated the reaction was complete. The reaction was cooled to room temperature, neutralized with saturated aqueous NaHCO<sub>3</sub> and extracted with CHCl<sub>3</sub>. The organic layer was washed with saturated aqueous NaCl, dried (MgSO<sub>4</sub>), filtered, concentrated and dried to afford 217 mg (96%) of 6-bromo-2,4-dimethyl-1H-benzimidazole. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 2.53 (s, 3 H), 2.61 (s, 3 H), 7.15 (s, 1 H), 7.47 (s, 1 H); MS ESI+ *m/z* 225 (M+H), 227 (M+H).

**Step 2: 2,4-Dimethyl-1H-benzo[d]imidazole-6-carboxylic acid (A10).** To a solution of 6-bromo-2,4-dimethyl-1H-benzimidazole (200 mg, 0.90 mmol) in degassed dioxane (2 mL) was added ((trans-bis(acetato)bis[o-(di-*o*-tolylphosphino)benzyl]-di-palladium(II))) (30 mg, 0.05 mmol), molybdenum hexacarbonyl (120 mg, 0.45 mmol) and a solution of Na<sub>2</sub>CO<sub>3</sub> (283 mg, 2.67 mmol) in degassed water (2.4 mL). The mixture was stirred for 20 sec and then heated at 165 °C for 15 min in a microwave reactor with absorption set at very high for 20 min. The reaction vessel was vented and filtered through diatomaceous earth. The solution was extracted with EtOAc and the aqueous phase was back extracted with additional EtOAc (2x). The combined organic extracts were set aside. Water (5 mL) was added to the aqueous extract which was then acidified with 0.5 M HCl to pH 3. The resulting solids were air dried to provide 97 mg (57%) of 2,4-dimethyl-1H-benzo[d]imidazole-6-carboxylic acid (A10). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 2.58 (s, 3 H), 2.63 (s, 3 H), 7.73 (s, 1 H), 8.04 (s, 1 H); MS ESI+ *m/z* 191.1 (M+H).

#### IV. Synthesis of Analogs 13-27

**2'-Ethyl-3'-methyl-1-(3-methyl-1H-indazole-5-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2-c]pyrazol]-7'(6'H)-one (13).** Compound **13** was prepared from **5d** and **A2** according to amidation method B (34% yield): <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ 0.65-0.97 (m, 1H), 0.97-1.44 (m, 2H), 1.96-2.38 (m, 1H), 2.67-2.87 (m, 1H), 3.06 (t, *J* = 7.06 Hz, 1H), 3.38 (br. s., 2H), 3.72 (t, *J* = 6.64 Hz, 2H), 6.02 (br. s., 1H), 7.28-7.41 (m, 1H), 7.40-7.54 (m, 2H), 7.66 (s, 1H), 7.73 (d, *J* = 7.48 Hz, 1H), 9.92 (s, 1H); MS ES+ *m/z* 408 (MH+); HPLC (Method LC-1) Retention time = 0.33 min.

**1-(3,7-Dimethyl-1H-indazole-5-carbonyl)-2'-ethyl-3'-methyl-2'H-spiro[piperidine-4,5'-pyrano[3,2-c]pyrazol]-7'(6'H)-one (14).** Compound **14** was prepared from **5d** and **A3** according to amidation method B (39% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.16 (t, *J* = 7.06 Hz, 1H), 1.38 (t, *J* = 7.27 Hz, 1H), 1.77 (br.s., 1H), 2.27 (s, 1H), 2.45-2.62 (m, 2H), 2.73 (br. s., 1H), 3.39-3.58 (m, 1H), 3.66 (br. s., 1H), 4.04-4.22 (m, 1H), 4.43 (br. s., 1 H), 4.89 (s, 2H), 7.21 (s, 1H), 7.65 (s, 1H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time = 0.38 min.



**2'-Ethyl-3'-methyl-1-(3-ethyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (15).** Compound **15** was prepared from **5d** and **A4** according to amidation method B (56% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.30-1.42 (m, 6 H), 1.79 (br. s., 3 H), 2.27 (s, 3 H), 2.74 (s, 3 H), 3.00 (q, *J* = 7.48 Hz, 2 H), 4.14 (q, *J* = 7.48 Hz, 2 H), 7.41-7.46 (m, 1H), 7.49-7.55 (m, 1H), 7.88 (s, 1 H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time= 0.39 min.

**2'-Ethyl-3'-methyl-1-(7-ethyl-1*H*-indazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (16).** Compound **16** was prepared from **5d** and **A5** according to amidation method B (64% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.24-1.46 (m, 6 H), 1.78 (br. s., 3 H), 2.27 (s, 4H), 2.74 (s, 3H), 2.97 (q, *J* = 7.48 Hz, 3H), 4.14 (q, *J* = 7.48 Hz, 3H), 7.25 (s, 2H), 7.72 (s, 2H), 8.11 (s, 1H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time = 0.39 min.

**1-(7-Ethoxy-3-methyl-1*H*-indazole-5-carbonyl)-2'-ethyl-3'-methyl-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (17).** Compound **17** was prepared from **5d** and **A6** according to amidation method B (43% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.38 (t, *J* = 7.27 Hz, 3 H), 1.50 (t, *J* = 7.06 Hz, 3 H), 1.79 (s, 2 H), 2.27 (s, 3 H), 2.52 (s, 3 H), 2.74 (s, 2 H), 4.14 (q, *J* = 7.27 Hz, 2 H), 4.24 (q, *J* = 7.06 Hz, 2 H), 6.83 (s, 1 H), 7.36 (s, 1 H); MS ES+ *m/z* 452 (MH+); HPLC (Method LC-1) Retention time = 0.47 min.

**1-(7-Ethoxy-3-ethyl-1*H*-indazole-5-carbonyl)-2'-ethyl-3'-methyl-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (18).** Compound **18** was prepared from **5d** and **A7** according to amidation method B (35% yield): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 1.29-1.55 (m, 5 H), 2.30 (s, 3 H), 2.58 (s, 3 H), 2.77 (br. s, 2 H), 2.92-3.11 (m, 2 H), 4.17 (q, *J* = 7.32 Hz, 2H), 7.24 (s, 1 H), 7.72 (s, 1 H); MS ES+ *m/z* 452 (MH+); HPLC (Method LC-1) Retention time = 0.39 min.

**2'-Ethyl-1-(1*H*-indazole-5-carbonyl)-3'-methyl-2'*H*-spiro[piperidine-4,5'-pyrano-[3,2-*c*]pyrazol]-7'(6'*H*)-one (19).** Compound **19** was prepared according from **5d** and 1*H*-indazole-5-carboxylic acid according to amidation method B (25% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.38 (t, *J* = 7.27 Hz, 3 H), 1.63-1.91 (m, 2 H), 1.91-2.24 (m, 2 H), 2.27 (s, 3 H), 2.74 (s, 2 H), 3.41-3.57 (m, 1 H), 3.67 (br.s., 1 H), 4.14 (q, *J* = 7.27 Hz, 2 H), 4.46 (br. S., 1H), 7.46 (d, *J* = 8.72 Hz, 1 H), 7.60 (d, *J* = 8.31 Hz, 1 H), 7.91(s, 1H), 8.12 (s, 1H); MS ES+ *m/z* 394 (MH+); HPLC (Method LC-1) Retention time = 0.33 min.

**1-(3,7-Dimethyl-1*H*-indole-5-carbonyl)-2'-ethyl-3'-methyl-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (20).** Compound **20** was prepared from **5d** and **A8** according to amidation method A (23% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.37 (t, *J* = 7.3 Hz, 3 H), 2.25 (s, 3 H), 2.28 (s, 3 H), 2.47 (s, 3 H), 2.73 (br.s., 2 H), 4.13 (q, *J* = 7.3 Hz, 2 H), 6.96 (s, 1 H), 7.05 (s, 1 H), 7.44 (s, 1H); MS ES+ *m/z* 421 (MH+); HPLC (method LC-2) Retention time = 2.2 min.

**2'-Ethyl-3'-methyl-1-(4-methyl-1*H*-benzo[*d*]imidazole-6-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2,*c*]pyrazol]-7'(6'*H*)-one (21).** Compound **21** was prepared from **5d** and **A9** according to amidation method A (25% yield): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 2.43-2.50 (m, 2 H), 2.54-2.60 (m, 2 H), 2.60-2.65 (m, 3 H), 4.80-4.89 (m, 1 H), 7.16 (br. s., 1 H), 7.53-7.55 (m, 2 H), 8.27 (s, 1 H); MS ES+ *m/z* 408 (MH+); HPLC (Method LC-1) Retention time = 0.41 min.

**1-(2,4-Dimethyl-1*H*-benzo[*d*]imidazole-6-carbonyl)-2'-ethyl-3'methyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2,*c*]pyrazol]-7'(6'*H*)-one (22).** Compound **22** was prepared from **5d** and **A10** according to amidation method B (26% yield): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 1.41 (t, *J* = 7.32 Hz, 3 H), 2.30 (s, 3 H), 2.57 (s, 3 H), 2.61 (s, 3 H), 2.76 (br. s., 2 H), 4.17 (q, *J* = 7.32 Hz, 2 H), 7.10 (s, 1 H), 7.42 (br. s., 1 H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time = 0.29 min.

**1-(3,7-Dimethyl-1*H*-indazole-5-carbonyl)-2'-isopropyl-2'*H*-spiro[piperidine-4,5'-pyrano[3,2,*c*]pyrazol]-7'(6'*H*)-one (23).** Compound **23** was prepared from **7d** and **A3** according to amidation method B (41% yield): <sup>1</sup>H NMR (400MHz, CD<sub>3</sub>OD) δ 1.10-1.20 (m, 1H), 1.38-1.51 (m, 2H), 2.50-2.57 (m, 6H), 2.70-2.79 (m, 2H), 3.42-3.52 (m, 2H), 4.45-4.55 (m, 1H), 7.20 (s, 1H), 7.49 (s, 1H), 7.65 (s, 1H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time = 0.46 min.

**3'-Methyl-1-(3,7-dimethyl-1*H*-indazole-5-carbonyl)-2'-(tetrahydrofuran-3-yl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2,*c*]pyrazol]-7'(6'*H*)-one (24).** Compound **24** was prepared from 2'-(Tetrahydrofuran-3-yl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2-*c*]pyrazol]-7'(6'*H*)-one (prepared as described in Corbett, J. W.; Elliot, R. L., Freeman-Cook, K. D., Griffith, D. A., Phillion, D. P. Spiro[pyrazolopyran-piperidine] ketones as acetyl-CoA carboxylase inhibitors and their preparation, pharmaceutical compositions and use in the treatment of diseases. PCT Int. Appl. 3 December 2009, WO 2009/144554) and **A3** according to amidation method A (56% yield): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.25 (br. s, 2 H), 1.67 (br. s, 2 H), 2.12 (br. s, 2 H), 2.27 (s, 3H), 2.30-2.42 (m, 2 H), 2.52 (s, 3 H), 2.57 (s, 3H), 2.69 (s,2H), 3.38 (br. s, 2 H), 3.78 (s,1H), 3.90-4.03 (m, 1H), 4.09-4.20 (s, 3H), 4.80-4.90 (m, 1H), 7.20 (s, 1 H), 7.60 (s, 1 H); MS ES+ *m/z* 464 (MH+); HPLC (Method LC-1) Retention time = 0.35 min.

**2'-(*tert*-Butyl)-1-(7-methyl-1*H*-benzo[*d*]imidazole-5-carbonyl)-2'*H*-spiro[piperidine-4,5'-pyrano[3,2,*c*]pyrazol]-7'(6'*H*)-one (25).** Compound **25** was prepared from **12d** and **A9** according to amidation method A (15% yield): <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 1.50 (d, *J* = 6.59 Hz, 6H), 2.60 (s, 3 H), 2.79 (s, 2 H), 4.51-4.57 (m, 1H), 7.52 (d, *J* = 1.22 Hz, 1H), 7.52 (s, 1 H), 7.82 (d, *J*=1.22 Hz, 1H); MS ES+ *m/z* 422 (MH+); HPLC (Method LC-1) Retention time = 0.32 min.

**2'-(tert-Butyl)-1-(2,7-dimethyl-1H-benzo[d]imidazole-5-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2,c]pyrazol]-7'(6'H)-one (26).** Compound **26** was prepared from **12d** and **A10** according to amidation method B (63% yield): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 1.57 (s, 9 H), 2.54 (s, 3 H), 2.57 (s, 3 H), 2.75 (br. s., 2 H), 7.06 (s, 1 H), 7.38 (br. s., 1 H), 7.58 (s, 1 H); MS ES+ *m/z* 436 (MH+); HPLC Method LC-1 Retention time = 0.33 min.

**2'-(tert-Butyl)-1-(1-methyl-1H-indazole-6-carbonyl)-2'H-spiro[piperidine-4,5'-pyrano[3,2,c]pyrazol]-7'(6'H)-one (27).** Compound **27** was prepared from **12d** and 1-methyl-1H-indazole-6-carboxylic acid according to amidation method A (58% yield): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.61 (s, 9 H), 1.75 (br. s., 1 H), 1.96 - 2.33 (comp, 2 H), 2.71 (s, 2 H), 3.24 - 3.74 (comp, 4 H), 4.10 (s, 3 H), 4.50 (br. s., 1 H), 7.14 (dd, J=8.30, 1.17 Hz, 1 H), 7.25 (s, 1 H), 7.53 (d, J= 1.17 Hz, 1 H), 7.75 (dd, J = 8.30, 0.8 Hz, 1 H), 8.01 (d, J=0.8 Hz, 1 H). MS ES+ *m/z* 408 (MH+); HPLC (Method LC-1) Retention time = 0.38 min.

## V. Malonyl CoA In Vivo Assay Description

*In vivo Study Design:* Male SD rats were acclimated for 7 days and prior to the study they were randomized on body weight (~200 g) into groups (n=8). The animals were orally dosed 2 hr into the light cycle with compound solutions prepared in (0.5% MC:0.1% PS80) with a dosing volume of 5 ml/kg. One hour post dose the animals were sacrificed via CO<sub>2</sub> asphyxiation followed by cervical dislocation. Blood for plasma exposure was collected, in EDTA, via cardiac puncture. Following centrifugation plasma was stored at -20°C pending compound exposure determinations. Liver and quadriceps were rapidly removed, freeze-clamped in a Wollenberg clamp (pre-cooled in liquid N<sub>2</sub>) and subsequently stored at -80°C.

*Extraction of tissue for MCoA determinations:* Frozen tissues were rapidly pulverized under liquid N<sub>2</sub> to maintain integrity of MCoA. Approximately 200 mg of pulverized tissue was added to a lysing matrix tube and immediately extracted in 10% TCA (1:5; tissue wt to acid vol) using a Fast Prep FP-120 (MP Bio). The extracted samples were centrifuged at 4°C (20000 x g) for 15 minutes (liver) and 30 minutes (muscle). After centrifugation the cleared supernatant was transferred to a 96 well polypropylene plate and store at -20°C until LC-MS analysis.

### *Quantitation of Tissue Malonyl CoA*

*Reagents:* Malonyl-CoA tetralithium salt and malonyl-<sup>13</sup>C<sub>3</sub>-CoA trilithium salt were purchased from Isotec (Miamisburg, OH, USA). Strata-X on-line solid phase extraction columns, 25 μm, 20 mm x 2.0 mm I.D (cat no. 00M-S033-B0-CB) were obtained from Phenomenex (Torrance, CA, USA). SunFire C18 reversed-phase columns, 3.5 μm, 100 mm x 3.0 mm I.D. (cat no.186002543) were purchased from Waters Corporation (Milford, MA, USA).

*Instrumentation and analysis:* The two-dimensional chromatographic system was equipped with Agilent 1100 quaternary and binary pumps and two Valco Cheminert 6-port two-position valves. Samples were introduced via a LEAP HTC PAL auto sampler with Peltier cooled stack maintained at 10°C and a 20 μL sampling loop. The analytical column was maintained at 35°C using a MicroTech Scientific Micro-LC Column Oven. An ABI Sciex API-3000 triple quadrupole mass spectrometer was used with a Turbo Ion Spray source. Two-dimensional

chromatography was performed with a Phenomenex Strata-X on-line solid phase extraction column utilizing a loading mobile phase of 100 mM sodium perchlorate / 0.1% (v/v) phosphoric acid (and methanol for washing/reconditioning) along with a Waters SunFire C18 reversed-phase column utilizing a gradient consisting of 100 mM ammonium formate/methanol to separate the malonyl CoA from isobaric 3-OH-butyryl CoA. An API-3000 mass spectrometer operating in positive ion mode (TurboIonSpray voltage = 2250V; nebulizing gas heated to 450°C.; DP, FP, CE set to 60, 340, 42 V, respectively; Q1 set to unit and Q3 set to low resolution; CAD gas = 8) was utilized to monitor MRM transitions for malonyl CoA ( $m/z$  854.1 $\rightarrow$ 347.1) and for malonyl- $^{13}\text{C}_3$ -CoA ( $m/z$  857.1 $\rightarrow$ 350.1)  $m/z$  with dwell times of 200 msec. Ion chromatograms were integrated using Analyst quantitation software (Applied Biosystems); peak area ratios (malonyl CoA/malonyl- $^{13}\text{C}_3$ -CoA) were compared against a standard curve prepared in 10% aqueous trichloroacetic acid to determine tissue concentrations of MCoA.