SUPPORTING INFORMATION

Structure-Activity Relationship Study of Indole-2-carboxamides Identifies a Potent Allosteric Modulator for the Cannabinoid Receptor 1 (CB1)

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CONTENT

Experimental section

Ethyl 5-chloro-3-pentanoyl-1*H*-indole-2-carboxylate (7a). 2.10 g (30.5 %) of the title compound was prepared from ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 5.0 g, 22.36 mmol) and valeroyl chloride (**6a**, 2.64 mL, 22.35 mmol) according to the general procedure A. The product was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) as a white solid. mp: 105-107 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-*d*): δ 9.12 (bs, 1H), 7.96 (dd, *J* = 1.8 Hz, 0.9 Hz, 1H), 7.42-7.28 (m, 2H), 4.46 (q, *J* = 7.2 Hz, 2H), 3.07 (t, *J* = 7.5 Hz, 2H), 1.77-1.67 (m, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.45-1.33 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H). MS (EI): *m/z* = 307.0 (M^{+}).

Ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate (7b). The title compound was prepared from ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 2.0 g, 8.94 mmol) and benzoyl chloride (**6b**, 1.03 mL, 8.94 mmol) according to the general procedure A. 1.2 g (41%) of light-yellow colored solid product was isolated. mp: 164–166 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.24 (bs, 1H), 7.87 (dd, *J* = 8.4 Hz, 1.1 Hz, 2H), 7.74 (d, *J* = 1.0 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.45 (m, 3H), 7.36 (dd, *J* = 8.5 Hz, 1.8 HZ, 1H), 3.98 (q, *J* = 7.1 Hz, 2H), 0.80 (t, *J* =7.1 Hz, 3H); MS (EI): *m/z* = 327.0 (M^{+}).

Ethyl 5-chloro-3-(cyclohexanoyl)-1*H*-indole-2-carboxylate (7c). The title compound was prepared from ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 2.0 g, 8.94 mmol) and cyclohexanecarbonyl chloride (**6c**, 1.19 mL, 8.94 mmol) according to the general procedure A. The yielded crude product was recrystallized in a mixture of ethyl acetate and acetone (6:1) and provided 510 mg crystals. The mother solution was concentrated and purified by Combiflash

chromatography (0-30% of ethyl acetate in hexane) to yield 495 mg light-yellow colored solid, which was then recrystallized in a hot mixture of ethyl acetate and acetone (6:1) to yield 245 mg crystals. The mother solution was again recrystallized in a hot mixture of ethyl acetate and acetone (8:1) and provided 161 mg of light-yellow colored solid product. Thus, a total of 916 mg of light-yellow colored crystal was collected as product (30.7 %). mp: 192–194 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.06 (s, 1H), 7.77 (s, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.33-7.31 (m, 1H), 4.48 (q, *J* = 7.2 Hz, 2H), 3.24 (tt, *J* = 12.5 Hz, 3.2 Hz, 1H), 1.91 (d, *J* = 13 Hz, 2H), 1.81 (dd, *J* = 9 Hz, 3.2 Hz, 2H), 1.72 (bs, 1H), 1.56-1.49 (m, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.35-1.27 (m, 3H). MS (EI): *m/z* = 333.1 (M^+).

Ethyl 5-chloro-3-heptanoyl-1*H*-indole-2-carboxylate (**7d**). The title compound (1.29 g, 43%) was prepared from ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 2.0 g, 8.94 mmol) and heptanoyl chloride (**6d**, 1.33 g, 8.94 mmol) according to the general procedure A. The product was isolated by Combiflash chromatography (0-30% ethyl acetate in hexane) as a white solid. mp: 114-115 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.09 (bs, 1H), 7.97 (s, 1H), 7.35 (d, *J* = 9.0 Hz, 1H), 7.32 (dd, *J* = 9.0 Hz, 1.2 Hz, 1H), 4.47 (q, *J* = 7.0 Hz, 2H), 3.06 (t, *J* = 7.5 Hz, 2H), 1.73 (p, *J* = 7.5 Hz, 2H), 1.44 (t, *J* = 7.0 Hz, 3H), 1.40-1.22 (m, 6H), 0.88 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 335.0 (M^+).

Ethyl 5-chloro-3-nonanoyl-1*H*-indole-2-carboxylate (7e). The title compound (1.05 g, 32 %) was synthesized from ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 2.0 g, 8.94 mmol) and nonanoyl chloride (**6e**, 1.61 mL, 8.94 mmol) according to the general procedure A. The product was isolated by Combiflash chromatography (0-40% ethyl acetate in hexane) as a white solid. mp: 101-102 ^{o}C. 1H NMR (500 MHz, Chloroform-*d*): δ 9.09 (bs, 1H), 7.97 (d, *J* = 1.0 Hz, 1H), 7.35 (d, *J* = 10 Hz, 1H), 7.32 (dd, *J* = 10 Hz, 1.0 Hz, 1H), 4.46 (q, *J* = 7.0 Hz, 2H), 3.03 (t, *J* = 7.6 Hz, 2H), 1.73 (p, *J* = 7.4 Hz, 2H), 1.44 (t, *J* = 7.2 Hz, 3H), 1.40-1.20 (m, 10H), 0.87 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 363.1 (M^+).

Ethyl 3-acetyl-5-chloro-1*H*-indole-2-carboxylate (7f). The commercially available ethyl 5-chloro-1*H*-indole-2-carboxylate (**5**; 3.0 g, 13.4 mmol) was acylated by acetyl chloride (**6f**, 1.21 g, 15.4 mmol) according to the general procedure A. The title compound was obtained as a white solid (1.58 g, 44.4 %). mp: 144-145 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 9.14 (bs, 1H), 8.12 (s, 1H), 7.35 (d, *J* = 10 Hz, 1H), 7.34-7.31 (m, 1H), 4.49 (q, *J* = 7.1 Hz, 2H), 2.74 (s, 3H), 1.45 (t, *J* = 7.1 Hz, 3H); MS (EI): (*m/z*) = 265.0 (M^+).

Ethyl 5-chloro-3-pentyl-1*H*-indole-2-carboxylate (8a). The title compound 1.73 g (90.6 %) was prepared from ethyl 5-chloro-3-pentanoyl-1*H*-indole-2-carboxylate (**7a**, 2.0 g, 6.50 mmol) according to the general procedure B. The product was isolated by Combiflash chromatography (0-20% ethyl acetate in hexane) as a white solid. mp: 105-107 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-*d*): δ 8.73 (bs, 1H), 7.64 (s, 1H), 7.31-7.24 (m, 2H), 4.44 (q, *J* = 7.5 Hz, 2H), 3.04 (t, *J* = 7.5 Hz, 2H), 1.72-1.60 (m, 2H), 1.50-1.31 (m, 7H, specially, 1.43 (t, *J* = 7.5 Hz, 3H), 0.90 (t, *J* = 7.5 Hz, 3H). MS (EI): *m/z* = 293.1 (M^+).

Ethyl 3-benzyl-5-chloro-1*H*-indole-2-carboxylate (8b). The title compound was prepared from ethyl 3-benzoyl-5-chloro-1*H*-indole-2-carboxylate (**7b**; 1.06 g, 3.23 mmol) according to the general procedure B. The crude product (1.76 g) was triturated with ethyl ether, ethyl acetate and

acetone successively and yielded 894 mg of off-white solid product, which was recrystallized in the mixture of ethyl acetate and acetone (4:1) to yield 440 mg of crystalline product. The mother solution was concentrated and recrystallized in a mixture of ethyl acetate and acetone (4:1) to yield 217.5 mg of crystals. All mother solutions were combined and concentrated and then purified by Combiflash chromatography (0-30% ethyl acetate in hexane) and yielded 120 mg of white crystals. A total of 777.5 mg (76.7 %) of product was obtained. mp: 175-177 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.72 (bs, 1H), 7.58 (dd, *J* = 1.8 Hz, 1 Hz, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 7.29-7.25 (m, 5H). 7.20-7.16 (m, 1H), 4.47 (s, 2H), 4.41 (q, *J* = 7.5 Hz, 2H), 1.38 (t, *J*= 7.5 Hz, 3H). MS (EI): *m/z* = 313 (M^{+}).

Ethyl 5-chloro-3-(cyclohexylmethyl)-1*H*-indole-2-carboxylate (8c) The title compound was synthesized from ethyl 5-chloro-3-(cyclohexanoyl)-1*H*-indole-2-carboxylate (**7c**; 905 mg, 2.71 mmol) according to the general procedure B. 638 mg (73.6 %) of white solid was isolated by Combiflash chromatography (0-30% ethyl acetate in hexane) as product. mp: 160-162 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.73 (bs, 1H), 7.62 (s, 1H), 7.17-7.24 (m, 2H), 4.41 (q, *J* = 7.2 Hz, 2H), 2.92 (d, *J* = 7.0 Hz, 2H), 1.69-1.60 (m, 6H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.20-1.0 (m, 5H). MS (EI): *m/z* = 319.1 (M^{+}).

Ethyl 5-chloro-3-heptyl-1*H*-indole-2-carboxylate (**8d**). The title compound (0.64 g, 66.7 %) was prepared from reduction of ethyl 5-chloro-3-heptanoyl-1*H*-indole-2-carboxylate (**7d**; 1.0 g, 2.98 mmol) according to the general procedure B. The product was isolated by Combiflash chromatography (0-30% ethyl acetate in hexane) as a white solid. mp: 91-92 ^{o}C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.71 (bs, 1H), 7.64 (s, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.25 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 3.03 (t, *J* = 7.6 Hz, 2H), 1.64 (p, *J* = 7.5 Hz, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.40-1.28 (m, 8H), 0.88 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 321.0 (M^{+}).

Ethyl 5-chloro-3-nonyl-1*H*-indole-2-carboxylate (8e). The title compound (880 mg, 85.5 %) was synthesized from ethyl 5-chloro-3-nonanoyl-1*H*-indole-2-carboxylate (**7e**; 1.07 g, 2.94 mmol) according to the general procedure B. The product was isolated by Combiflash chromatography (0-30% ethyl acetate in hexane) as a white solid. mp: 90-92 ºC. 1H NMR (500 MHz, Chloroform-*d*): δ 8.70 (bs, 1H), 7.64 (d, *J* = 1.0 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H), 7.25 (dd, *J* = 9.0 Hz, 1.0 Hz, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 3.03 (t, *J* = 7.5 Hz, 2H), 1.68-1.60 (m, 2H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.40-1.22 (m, 12H), 0.88 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 349.2 (M^{+}).

Ethyl 5-chloro-3-ethyl-1*H*-indole-2-carboxylate (8f). The title compound was prepared from ethyl 3-acetyl-5-chloro-1*H*-indole-2-carboxylate (**7f**; 1.0 g, 3.76 mmol) according to general procedure B to yield 0.85 g (89.8 %) of white solid product. mp: 108-110 ^{o}C. ^{1}H NMR (300 MHz, Chloroform-*d*): δ 8.71 (bs, 1H), 7.65 (s, 1H), 7.30 (dd, *J* = 10 Hz, 1.0 Hz, 1H), 7.28-7.25 (m, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.07 (q, *J* = 7.5 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.5 Hz, 3H). MS (EI): *m/z* = 251.1 (M^{+}).

5-Chloro-3-pentyl-1*H*-indole-2-carboxylic acid (9a). The title compound 1.28 g (83.2 %) was prepared from ethyl 5-chloro-3-pentyl-1*H*-indole-2-carboxylate (**8a**, 1.70 g, 5.79 mmol) according to the general procedure C. The product was yielded as a white solid. mp: 160-161 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.02 (bs, 1H), 11.57 (s, 1H), 7.67 (d, *J* = 2.0 Hz, 1H), 7.39

(d, *J* = 8.7 Hz, 1H), 7.22 (dd, *J* = 8.7, 2.0 Hz, 1H), 3.01 (t, *J* = 7.5 Hz, 2H), 1.65-1.47 (m, 2H), 1.37-1.25 (m, 4H), 0.85 (t, *J* = 7.5 Hz, 3H). MS (EI): *m/z* = 265.0 (M^+).

3-Benzyl-5-chloro-1*H*-indole-2-carboxylic acid (9b). The title compound (600 mg, 90.1 %) was prepared from hydrolysis of ethyl 3-benzyl-5-chloro-1*H*-indole-2-carboxylate (**8b**, 730 mg, 2.33 mmol) according to the general procedure C. The product was isolated as an off-white solid. mp: 263-265 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.24 (s, 1H), 11.74 (s, 1H), 7.61 (s, 1H), 7.41(d, *J* = 8.5 Hz, 1H), 7.28-7.20 (m, 5H), 7.12 (t, *J* = 7.5 Hz, 1H), 4.43 (s, 2H). MS (EI): *m/z* = 285.0 (M^+).

5-Chloro-3-(cyclohexylmethyl)-1*H*-indole-2-carboxylic acid (9c). The title compound was obtained from ethyl 5-chloro-3-(cyclohexylmethyl)-1*H*-indole-2-carboxylate (**8c**; 610 mg, 1.9 mmol) according to the general procedure C. 504 mg (90.4 %) of the product was isolated as white solid. mp: 220-222 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.99 (bs, 1H), 11.59 (s, 1H), 7.65 (s, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 7.23 (ddd, *J* = 8.9, 1.8, 0.9 Hz, 1H), 2.90 (d, *J* = 6.7 Hz, 2H), 1.64-1.58 (m, 6H), 1.20-0.9 (m, 5H). MS (EI): *m/z* = 291.1 (M^+).

Ethyl 5-chloro-3-heptyl-1*H*-indole-2-carboxylic acid (**9d**). The title compound (242 mg, 88.6 %) was obtained from hydrolysis of ethyl 5-chloro-3-heptyl-1*H*-indole-2-carboxylate (**8d**, 300 mg, 0.93 mmol) by following the general procedure C. The product was isolated as a white solid. mp: 135-136 °C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.74 (bs, 1H), 7.67 (s, 1H), 7.34-7.28 (m, 2H), 3.10 (t, *J* = 7.8 Hz, 2H), 1.64 (p, *J* = 7.5 Hz, 2H), 1.50-1.20 (m, 8H), 0.89 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 293.2 (M^+).

5-Chloro-3-nonyl-1*H*-indole-2-carboxylic acid (9e). The title compound (750 mg, 92.5 %) was prepared from ethyl-5-chloro-3-nonyl-1*H*-indole-2-caboxylate (**8e**; 880 mg, 2.52 mmol) according to the general procedure C. The product was isolated as a white solid. mp: 120-122 °C. 1H NMR (500 MHz, Chloroform-*d*): δ 8.77 (bs, 1H), 7.67 (s, 1H), 7.32 (d, *J* = 10 Hz, 1H), 7.30-7.28 (m, 1H), 3.09 (t, *J* = 7.5 Hz, 2H), 1.75-1.65 (m, 2H), 1.45-1.20 (m, 12H), 0.87 (t, *J* = 7.0 Hz, 3H). MS (EI): *m/z* = 321.2 (M^+).

5-Chloro-3-ethyl-1*H*-indole-2-carboxylic acid (9f). The title compound (550 mg, 83 %) was prepared from hydrolysis of ethyl 5-chloro-3-ethyl-1*H*-indole-2-carboxylate (**8f**, 750 mg, 2.97 mmol) according to the general procedure C. The product was obtained as a white solid. mp: 155-157 °C. ^{1}H NMR (500 MHz, Chloroform-*d*): δ 8.73 (bs, 1H), 7.89 (s, 1H), 7.33 (d, *J* = 8.7 Hz, 1H), 7.30 (dd, *J* = 8.7 Hz, 2.0 Hz, 1H), 3.13 (q, *J* = 7.5 Hz, 2H), 1.31 (t, *J* = 7.5 Hz, 3H). MS (EI): *m/z* = 223.0 (M^+).