Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Data

Discovery of Acrylonitrile-Based Small Molecules Active Against *Haemonchus contortus*

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General Experimental

1. General Chemistry

All reagents were purchased from Sigma-Aldrich, Matrix Scientific or Lancaster Synthesis and were used without purification. With the exception of THF (anhydrous > 99%) obtained from Sigma-Aldrich, all solvents were re-distilled from glass prior to use.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance™ AMX 300 MHz spectrometer at 300.1315 and 75.4762 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards, and coupling constants (*J*) are expressed in Hertz (Hz). Mass spectra were recorded on a Shimadzu LCMS 2010 EV using a mobile phase of 1:1 acetonitrile:H_2O with 0.1% formic acid.

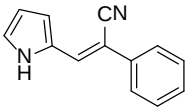
Melting points were recorded on a Stuart Scientific melting point apparatus (UK) and are uncorrected. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230-400 mesh) or using the Biotage SP4 flash purification system with a 100 g pre-packed snap column.

Microwave irradiations were conducted using a CEM Discover® BenchMate microwave (120°C), and hydrogenations were performed using the H-Cube® continuous-flow hydrogenation reactor.

2. Preparation of Propanenitrile Analogues and precusors (7a – 7d & 8a – 8d)

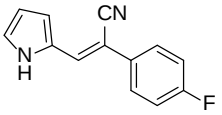
Note; experimental data for preparation of **7a** – **7d** refer to references 1-3

2-Phenyl-3-(1H-pyrrol-2-yl)acrylonitrile (7a)



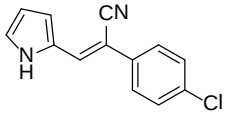
1*H*-pyrrole-2-carbaldehyde (165 mg, 1.74 mmol), was added to a vigorously stirred solution of water (10 mL) and heated to 50 °C upon which it dissolved. 2-Phenylacetonirile (193 mg, 1.65 mmol) was then slowly added forming a suspension. Once a clear solution was evident, typically 5-10 minutes, 40 % PhCH2NMe3(OH) (7 mL) was added dropwise. After complete addition, the reaction vessel was sealed and stirred at 50 °C for 5 hours. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a solid. The crude solid was then recrystallised from EtOH to afford **5a** as a brown solid; 73%; 94–96 ºC. ^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.81 (br, 1H, NH), 7.61-7.57 (m, 2H, Ar H2; Ar H6), 7.45-7.40 (m, 2H, Ar H3; Ar H5), 7.42 (s, 1H, HC=C), 7.35-7.30 (m, 1H, Ar H4), 7.08-7.06 (m, 1H, Pyr H-5), 6.73 (dd, *J* = 1.4, 3.7 Hz, 1H, Pyr H3), 6.37 (dd, *J* = 1.4, 3.7, 1H (Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 133.4, 130.7, 128.5 (2 x Ar), 127.6, 127.2, 124.4 (2 x Ar), 123.5, 120.1, 118.5, 110.3, 100.8; υmax(KBr)/cm-1: 3396 (NH), 2205 (CN), 1683 (C=C), 1601 (Ar), 1589 (Ar), 1496 (Ar); LRMS (APCI M+1) 195.

2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (7b)



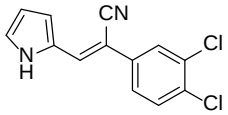
Synthesized using the general procedure as for 1, from 1H-pyrrole-2-carbaldehyde and 4-fluorophenylacetonitrile to afford 2 as a yellow solid; 78%; mp 115-116 ºC. ^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.82 (br, 1H, NH), 7.56-7.51 (m, 2H, Ar H2; Ar H6), 7.32 (s, 1H, HC=C), 7.13-7.06 (m, 3H, Ar H3; Ar H5; Pyr H5), 6.71-6.70 (m, 1H, Pyr H3), 6.36-6.34 (m, 1H, Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 130.7, 129.6, 127.0, 126.1 (2 x Ar), 123.5, 119.9, 118.5, 115.7, 115.4, 110.3 (2 x Ar), 99.7; υmax(KBr)/cm-1: 3401 (NH), 2205 (CN), 1641 (C=C), 1597 (Ar), 1507 (Ar); LRMS (APCI M+1) 213.

2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (7c)



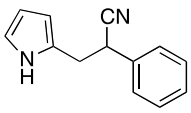
Synthesized using the general procedure as for 1, from 1H-pyrrole-2-carbaldehyde and 4-chlorophenylacetonitrile to afford 3 as a yellow solid; 67%; mp 112–114 ºC. ^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.78 (br, 1H, NH), 7.51-7.49 (m, 2H, Ar H2; Ar H6), 7.38-7.35 (m, 3H, Ar H3; Ar H5; HC=C), 7.08 (s, 1H, Pyr H5), 6.72 (d, J = 2.7 Hz, 1H, Pyr H3), 6.36 (s, 1H, Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 133.4, 131.9, 130.9, 128.7 (2 xAr), 126.9, 125.6 (2 x Ar), 123.8, 119.7, 118.9, 110.4, 99.5; υmax(KBr)/cm-1: 3380 (NH), 2213 (CN), 1636 (C=C), 1603 (Ar), 741 (Ar-Cl); LRMS (APCI M+1) 229.

2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)acrylonitrile (7d)



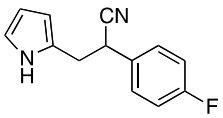
Synthesized using the general procedure as for 1, from 1H-pyrrole-2-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford 3 as a dark yellow solid; 72%; mp 140–142 ºC. ^{1}H NMR ($CDCl_3$) (300 MHz): δ 9.78 (br, 1H, NH), 7.88 (s, 1H, HC=C), 7.78 (d, J = 2.1 Hz, 1H, Ar H5), 7.64-7.56 (m, 2H, Ar H2; Ar H6), 7.26-7.24 (m, 1H, Pyr H5), 7.21-7.20 (m, 1H Pyr H3), 6.39-6.37 (m, 1H Pyr H4); ^{13}C NMR ($CDCl_3$) (75 MHz): δ 135.0, 132.7, 132.0, 130.5, 130.1, 127.1, 125.7, 124.1, 123.9, 117.8, 114.4, 110.8, 98.3; υmax KBr)/cm-1: 3415 (NH), 2199 (CN), 1636 (C=C), 1604 (Ar), 1588 (Ar); LRMS (APCI M+1) 263.

2-Phenyl-3-(1H-pyrrol-2-yl)propanenitrile (8a)



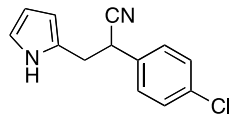
General Procedure 1: 2-Phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile (**7a**) (990 mg, 5.1 mmol) was dissolved into sufficient freshly distilled dry acetone to form a 0.05 M solution. This solution was hydrogenated using the ThalesNano H-cube™ using a 10% Pd/C catalyst at 1 mL/min at 50 ºC and 50 bar H_2 pressure. After completion of the reaction, the solvent was removed *in vacuo* and the resulting crude oil was subjected to flash silica chromatography (1:1 $CHCl_3$:Hexanes) to afford **6a** as a brown oil (980 mg, 98%); δ_H ($CDCl_3$) (300 MHz): 8.03 (br, 1H, NH), 7.42-7.35 (m, 3H, H-3, H-4 and H-5), 7.29-7.26 (m, 2H, H-2 and H-6), 6.69-6.67 (m, 1H, H-5′), 6.15-6.13 (m, 1H, H-3′), 6.03-6.02 (m, 1H, H-4′), 4.01 (t, *J* = 7.4 Hz, 1H, CH), 3.28-3.14 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 134.5, 128.6 (2 x Ar), 127.8, 126.8, 125.7 (2 x Ar), 120.4, 117.3, 108.1, 107.4, 38.4, 34.0; IR (film) ν_{max}/cm^{-1}: 3384 (NH), 2242 (CN), 1597 (Ar); m/z (APCI M+H) 197; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{12}N_2$; Exact Mass: 197.1079, found: 197.1083.

2-(4-Fluorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (8b*)*



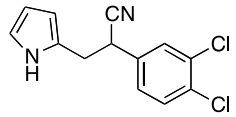
Synthesized using the general procedure as for **8a**, from (Z)-2-(4-fluorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**7b**) to afford **8b** as a light brown oil (95%); δ_H ($CDCl_3$) (300 MHz): 8.06 (br, 1H, NH), 7.24-7.19 (m, 2H, H-2 and H-5), 7.09-7.03 (m, 2H, H-3 and H-5), 6.69 (d, *J* = 1.4 Hz, 1H, H-5′), 6.15-6.12 (m, 1H, H-3′), 5.98 (s, 1H, H-4′), 4.00 (t, *J* = 6.8 Hz, 1H, CH), 3.25-3.14 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 163.6, 130.2, 128.6 (2 x Ar), 125.3, 120.2, 117.4, 115.4, 108.2 (2 x Ar), 107.6, 37.6, 34.0; IR (film) ν_{max}/cm^{-1}: 3404 (NH), 2244 (CN), 1602 (Ar), 1509 (Ar); m/z (APCI M+H) 215; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{11}FN$; Exact Mass: 215.0985, found: 215.0986.

2-(4-Chlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (8c)



Synthesized using the general procedure as for **8a**, from (*Z*)-2-(4-chlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile (**7c**) to afford **8c** as a light yellow oil (76%); δ_H ($CDCl_3$) (300 MHz): 8.04 (br, 1H, NH), 7.38-7.32 (m, 2H, H-3 and H-5), 7.19-7.16 (m, 2H, H-2 and H-6), 6.70-6.68 (m, 1H, H-5′), 6.15-6.12 (m, 1H, H-3′), 5.99-5.98 (m, 1H, H-4′), 3.99 (t, *J* = 6.7 Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 132.9, 128.7 (2 x Ar), 128.6, 128.2, 126.8 (2 x Ar), 229.9, 117.4, 108.2, 107.7, 37.8, 33.9; IR (film) ν_{max}/cm^{-1}: 3398 (NH), 2215 (CN), 1598 (Ar), 1511 (Ar); m/z (APCI M+H) 231; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{11}ClN_2$; Exact Mass: 231.0689, found: 231.0694.

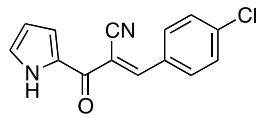
2-(3,4-Dichlorophenyl)-3-(1H-pyrrol-2-yl)propanenitrile (8d)



Synthesized using the general procedure as for **8a**, from (Z)-2-(3,4-dichlorophenyl)-3-(1*H*-pyrrol-2-yl)acrylonitrile **7d** to afford **8d** as a yellow oil (65%); δ_H ($CDCl_3$) (300 MHz): 8.10 (br, 1H, NH), 7.45-7.33 (m, 2H, H-2 and H-5), 7.07-7.04 (m, 1H, H-6), 6.72-6.70 (m, 1H, H-5′), 6.16-6.13 (m, 1H, H-3′), 5.98 (s, 1H, H-4′), 3.97 (t, *J* = 6.6Hz, 1H, CH), 3.25-3.12 (m, 2H, CH_2); δ_C ($CDCl_3$) (75 MHz): 134.4, 132.7, 132.2, 130.4, 128.8, 126.2, 124.7, 119.4, 117.6, 108.3, 107.9, 37.6, 33.7; IR (film) ν_{max}/cm^{-1}: 3392 (NH), 2221 (CN), 1600 (Ar); m/z (APCI M+H) 267; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{13}H_{10}Cl_2N_2$; Exact Mass: 265.0299, found: 265.0305.

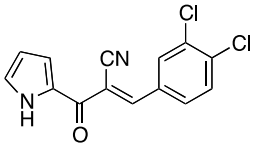
3. Preparation of 1*H*-pyrrole-β-oxo-2-propanenitrile Analogues (9a – 9e, 10)

3-(4-Dichlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (9a)



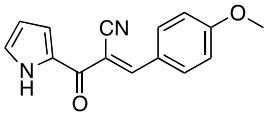
General Procedure 2: Cyanoacetic acid (1.36g, 16 mmol) was added to acetic anhydride (8 mL) and the resultant suspension was stirred and heated to 50 °C until complete dissolution was observed. Pyrrole (1.07g, 16 mmol) was subsequently added and the reaction mixture was heated at 75 °C for 35 minutes. After this time, EtOAc (20 mL) was added and the reaction mixture was washed with 0.1M NaOH (3 x 10 mL). The organic layer was collected, dried ($MgSO_4$) and evaporated under reduced pressure. The resulting residue was purified by flash silica chromatography (1:10 EtOAc:Hexanes to 1:1 EtOAc:Hexanes) to afford 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (**10**) (1.50 g, 70%). This freshly prepared 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile (**10**) (200 mg, 1.49 mmol) in EtOH (10 mL) was added a solution of 4-chlorobenzaldehyde (220 mg, 1.57 mmol) in EtOH (10 mL). Stirring was continued and the reaction mixture was heated to 70 ºC and piperidine (2 drops) was added. After addition of the piperidine, the reaction mixture was heated under reflux for 2 hours. After this time, the reaction mixture was cooled and the solvent removed *in vacuo* to afford a brown oil which was purified by flash chromatography (1:10 EtOAc:Hexanes) to afford **9a** as a yellow solid (39%), m.p. 192-194 °C; δ_H (Acetone-d_6) (300 MHz): 11.30 (br, NH), 8.28 (s, 1H, CH=C), 8.13-8.11 (m, 2H, H-2 and H-6), 7.66-7.63 (m, 2H, H-3 and H-5), 7.45-7.44 (m, 1H, H-5′), 7.35-7.34 (m, 1H, H-3′), 6.38-6.36 (m, 1H, H-4′); δ_C (Acetone-d_6) (75 MHz): 173.6, 151.1, 137.4, 131.7 (2 x Ar), 130.8, 128.8 (2 x Ar), 126.9, 119.7, 118.7, 116.6, 110.4, 109.5; IR (KBr) ν_{max}/cm^{-1}: 3284 (NH), 2211 (CN), 1627 (C=O); m/z (APCI M+H) 257; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_9ClN_2O$; Exact Mass: 256.0403, found: 257.0478.

3-(3,4-Dichlorophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (9b)



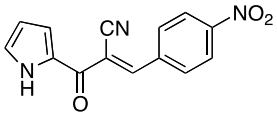
Synthesized using the general procedure 2, 3-oxo-3-(1H-pyrrol-2-yl)propanenitrile and 3,4-dichlorobenzaldehyde to afford **9b** as a yellow solid (66%), m.p. 178-181 °C; δ_H (Acetone-d_6) (300 MHz): 11.31 (br, NH), 8.29-8.27 (m, 2H, H-5 and CH=C), 8.11-8.08 (m, 1H, H-6), 7.84-7.81 (m, 1H, H-2), 7.45-7.36 (m, 2H, H-5′ and H-3′), 6.39-6.37 (m, 1H, H-4′); δ_C (Acetone-d_6) (75 MHz): 173.4, 149.6, 135.0, 132.4, 132.1, 131.7, 130.8, 129.3, 127.2, 127.0, 119.0, 116.3, 111.0, 110.5; IR (KBr) ν_{max}/cm^{-1}: 3310 (NH), 2222 (CN), 1632 (C=O); m/z (APCI M+H) 290; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_8Cl_2N_2O$; Exact Mass: 290.0014, found: 291.0079.

3-(4-Methoxyphenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (9c)



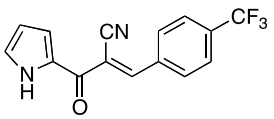
Synthesized using the general procedure 2, 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-methoxybenzaldehyde to afford **9c** as a yellow solid (83%), m.p. 166-168 °C; δ_H (DMSO-d_6) (300 MHz): 12.14 (br, NH), 8.24 (s, 1H, CH=C), 8.08 (d, *J* = 8.9Hz, 2H, H-2 and H-6), 7.28-7.25 (m, 2H, H-5′ and H-3′), 7.13 (d, *J* = 8.9Hz, 2H, H-3 and H-5), 6.31 (s, 1H, H-4′), 3.85 (s, 3H, OCH_3); δ_C (DMSO-d_6) (75 MHz): 174.9, 163.0, 153.0, 133.1 (2 x Ar), 129.1, 127.5, 124.6, 118.9, 118.2, 114.8 (2 x Ar), 110.6, 105.5, 55.6; IR (KBr) ν_{max}/cm^{-1}: 3306 (NH), 2209 (CN), 1617 (C=O), 1507 (Ar); m/z (APCI M+H) 253; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{15}H_{12}N_2O_2$; Exact Mass: 252.0899, found: 253.0979.

3-(4-Nitrophenyl)-2-(1H-pyrrole-2-carbonyl)acrylonitrile (9d)



Synthesized using the general procedure 2, 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-nitrobenzaldehyde to afford **9d** as a purple solid (37%), m.p. 199-200 °C; δ_H (DMSO-d_6) (300 MHz): 12.32 (br, NH), 8.40-8.38 (m, 3H, H-3, H-5 and CH=C), 8.24-8.21 (m, 2H, H-2 and H-6), 7.24 (m, 2H, H-5′ and H-3′), 6.34 (s, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.2, 150.6, 148.8, 138.2, 131.3 (2 x Ar), 130.6, 128.7, 124.0 (2 x Ar), 120.3, 116.6, 113.1, 111.1; IR (KBr) ν_{max}/cm^{-1}: 3308 (NH), 2228 (CN), 1633 (C=O), 1517 (NO) 1343 (NO); m/z (APCI M+H) 268; HRMS (APCI M+H): Calculated for Chemical Formula: $C_{14}H_9N_3O_3$; Exact Mass: 267.0644, found: 268.0717.

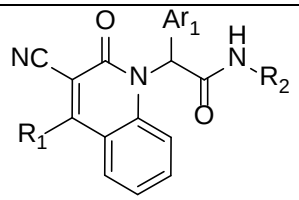
2-(1H-Pyrrole-2-carbonyl)-3-(4-(trifluoromethyl)phenyl)acrylonitrile (9e)

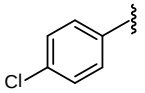
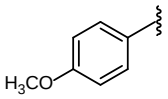
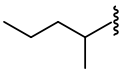
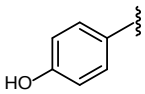
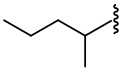
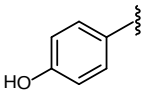
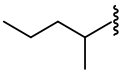
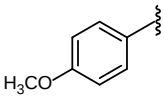
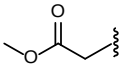
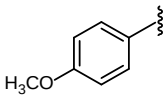
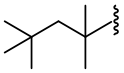
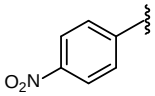
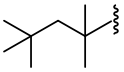
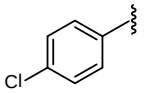
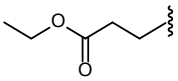
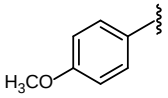
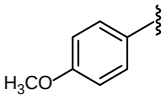
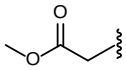
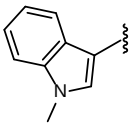
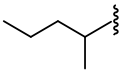
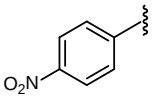
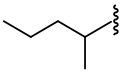


Synthesized using the general procedure 2, 3-oxo-3-(1*H*-pyrrol-2-yl)propanenitrile and 4-(trifluoromethyl)benzaldehyde to afford **9e** as a yellow solid (41%), m.p. 160-162 °C; δ_H (DMSO-d_6) (300 MHz): 12.30 (br, 1H), 8.37 (s, 1H, CH=C), 8.19 (d, *J* = 8.1Hz, 2H, H-2 and H-6), 7.94 (d, *J* = 8.1Hz, 2H, H-3 and H-6), 7.32 (s, 2H, H-5′ and H-3′), 6.34 (s, 1H, H-4′); δ_C (DMSO-d_6) (75 MHz): 174.4, 151.4, 136.0, 131.2, 130.8 (2 x Ar), 128.8, 128.5 (2 x Ar), 125.9, 125.8, 120.1, 116.8, 112.1, 111.0; IR (KBr) ν_{max}/cm^{-1}: 3295 (NH), 2228 (CN), 1637 (C=O), 1561 (Ar), 1325 (C-F); m/z (ESI M-H) 289; HRMS (ESI M-H): Calculated for Chemical Formula: $C_{15}H_9F_3N_2O$; Exact Mass: 290.0667, found: 289.0673.

4. Structure of quninolin-2-(1H)-ones subjected to the H. contortus larval development assay (LDA) (11a-l).

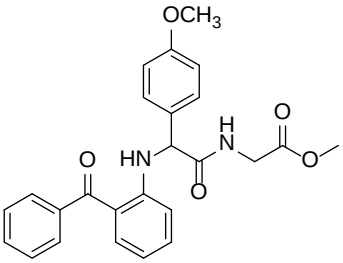
*Note: for all experimental data relating to compounds **9a-l** refer to reference [4].*



Compound	R_1	Ar_1	R_2	LD_{50} (μM)
11a				> 100
11b				> 100
11c				> 100
11d				> 100
11e				> 100
11f				> 100
11g				> 100
11h				> 100
11i				> 100
11j				> 100
11k				> 100
11l				> 100

5. Preparation of α-amino amides

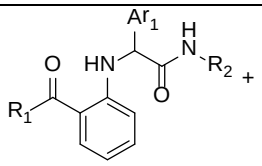
Methyl-2-(2-(2-benzoylphenylamino)-2-(4-methoxyphenyl)acetamido)acetate (12a)

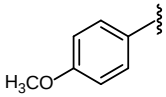
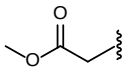
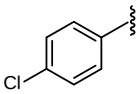
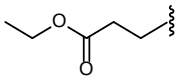
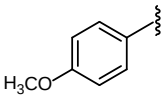
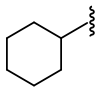
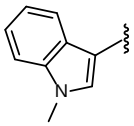
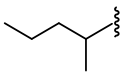
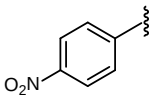
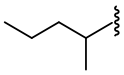
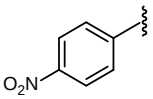
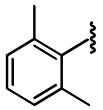
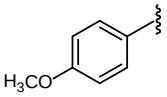
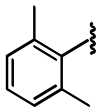
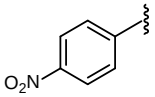
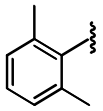
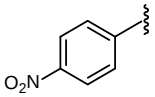
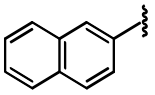
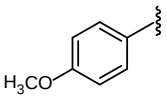
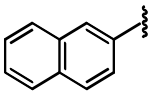
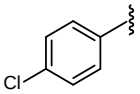
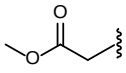
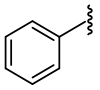
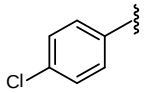
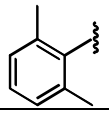


General Procedure 3: Compound **12a** was synthesised utilising the previously reported procedure[4] using 2-aminobenzophenone (0.20 g, 1.00 mmol), 4-methoxybenzaldehyde (0.12 mL, 1.00 mmol), cyanoacetic acid (0.09 g, 1.00 mmol), methylisocyanoacetate (0.10 mL, 1.00 mmol), and MeOH (5.0 mL). The crude reaction material was subjected to flash silica gel column chromatography (1:1 Hexanes:EtOAc) affording **12a** (0.17 g, 35%) as a yellow oil. ^{1}H NMR (300 MHz) (Acetone-d_6) δ 9.35 (1 H, d, *J* = 6.1 Hz), 7.95 (1 H, t, *J* = 5.9 Hz), 7.62-7.47 (9 H, m), 7.34 (1 H, t, *J* = 8.5 Hz), 6.94 (2 H, d, *J* = 8.9 Hz), 6.76 (1 H, d, *J* = 8.9 Hz), 6.61 (1 H, t, *J* = 7.5 Hz), 5.46 (1 H, d, *J* = 6.1 Hz), 3.95 (2 H, abq, *J* = 18.0, 6.0 Hz), 3.78 (3 H, s), 3.62 (3 H, s); ^{13}C NMR (300 MHz) (Acetone-d_6) δ 197.9, 170.4, 169.3, 159.1, 149.1, 139.8, 134.2, 134.0, 130.4, 130.3, 128.4, 128.0 (2C), 127.5, 117.8, 114.2, 113.6 (2C), 112.3, 60.0, 54.1, 50.7, 40.3; MS (ESI$^+$) *m/z* 433 (M+1, 100%); HRMS (ESI$^+$) for $C_{25}H_{25}N_2O_5$, calculated 433.1685 found 433.1687.

5.1 Structure of α-amino amides subjected to the H. contortus larval development assay (LDA) (10al)

*Note: for all experimental data relating to compounds **10a-l** refer to reference 4*

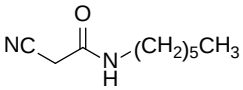


Compound	R_1	Ar_1	R_2	LD_{50} (μM)
12a				< 10
12b				> 100
12c				> 100
12d				> 100
12e				> 100
12f				> 100
12g				> 100
12h				> 100
12i				> 100
12j				> 100
12k				> 100
12l				> 100

6. Preparation of the Cyanoamide Dynole™ Series (13a – c, 16, 19 - 22)

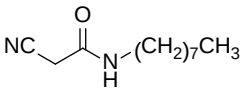
6.1 Preparation of Cyanoamides Required for Compounds 13a-c, 19 - 22 (compounds 15a-c)

2-cyano-*N*-hexylacetamide (15a)



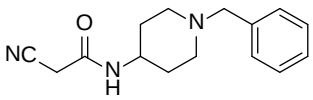
General Procedure 4: A solution of methyl cyanoacetate (2.16 g, 21.8 mmol), n-hexylamine (2.83 g, 21.8 mmol), and MeOH (10 mL) were stirred at rt for 4 h. The resulting solid was collected by vacuum filtration and recrystallised from EtOH to afford 2-cyano-*N*-octylacetamide (3.38 g, 79%) as a white flaky solid (m.p. 68-70°C). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 6.48 (1H, bs), 3.37 (2H, s), 3.25 (2H, q, *J* = 6.6 Hz), 1.51 (2H, quin, *J* = 6.6 Hz), 1.28 (6 H, m), 0.86 (3H, t, *J* = 6.3 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 160.6, 114.4, 39.8, 31.2, 28.9, 28.6, 28.5, 26.2, 13.9; MS (ES+) *m/z* 169 (M+1, 100%).

2-cyano-N-octylacetamide (15b)



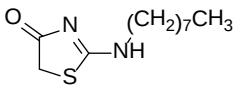
Synthesized using general procedure 4 whereby a solution of methyl cyanoacetate (2.16 g, 21.8 mmol), n-octylamine (2.83 g, 21.8 mmol), and MeOH (10 mL) were stirred at rt for 4 h. The resulting solid was collected by vacuum filtration and recrystallised from EtOH to afford 2-cyano-*N*-octylacetamide (3.38 g, 79%) as a white flaky solid (m.p. 68-70°C). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 6.48 (1H, bs), 3.37 (2H, s), 3.25 (2H, q, *J* = 6.6 Hz), 1.51 (2H, quin, *J* = 6.6 Hz), 1.28 (10 H, m), 0.86 (3H, t, *J* = 6.3 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 160.6, 114.4, 39.8, 31.2, 28.9, 28.6, 28.5, 26.2, 25.3, 22.0, 13.9; MS (ES+) *m/z* 197 (M+1, 100%)

***N*-(1-benzylpiperidin-4-yl)-2-cyanoacetamide (15c)**



Synthesized using general procedure 4 and recrystallisation from EtOH afforded **11c** as an off-white crystalline solid (38%), m.p. 178 °C. ^{1}H NMR (300 MHz) (MeOD) δ 7.63-6.98 (6 H, m), 4.86 (2 H, s), 3.66 (1 H, ddd, *J* = 15.1, 10.8, 4.2 Hz), 3.53 (2 H, s), 2.87 (2 H, m), 2.15 (2 H, dt, *J* = 11.8, 11.8, 1.9 Hz), 1.94-1.79 (2 H, m), 1.52 (2 H, dq, *J* = 11.9, 11.9, 11.8, 3.7 Hz); ^{13}C NMR (300 MHz) (MeOD) δ 162.0, 136.3, 128.8, 127.4, 126.6, 114.1, 61.9, 51.1, 46.6, 46.5, 30.1; MS (ES+) *m/z*: 258.2 (M+1, 100%); HRMS calculated for $C_{15}H_{19}N_3O$ Exact Mass: 257.1528; Elemental Analysis: C, 70.01; H, 7.44; N, 16.33; O, 6.22.

2-(octylamino)thiazol-4(5H)-one (*Precursor for compound 19*)

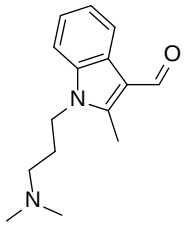


To a stirred solution of 2-thio-4-thiazolin-4-one (0.82 g, 6.2 mmol), 2-octylamine (1.59 g, 12.4 mmol) and acetonitrile (15 mL) was added *N*,*N*-diisopropylethylamine (0.72, 5.5 mmol). The mixture was cooled to 0 °C prior to the addition of $MgCl_2$ (1.70 g, 6.2 mmol). The resulting solution was stirred at r.t. for 48 h, filtered through celite, concentrated *in vacuo*, dried ($MgSO_4$), and subjected to flash silica column chromatography (1:1 Hexanes:EtOAc) to afford 2-(octylamino)thiazol-4(5H)-one (0.71 g, 51%) as a yellow solid (mp 134 °C); ^{1}H NMR (300 MHz) (MeOD) δ 5.74 (2 H, s), 4.34 (1 H, t, *J* = 7.1 Hz), 2.49 (2 H, qd, *J* = 17.7, 7.0 Hz), 2.16 (12 H, m), 1.76 (3 H, t, *J* = 6.6 Hz); ^{13}C NMR (75 MHz) (MeOD) δ 188.8, 181.4, 44.5, 37.8, 31.0, 28.4, 28.3, 28.0, 25.8, 21.7, 12.5;

MS (ES+) *m/z* 229 (M+1, 100%)

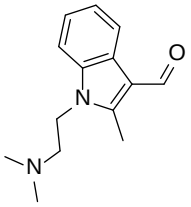
6.2 Preparation of the Aldehydes Required for Synthesis 13a-13c 19-22

1-(3-(dimethylamino)propyl)-1H-indole-3-carbaldehyde (*Precursor for compound 13a and 13c)*



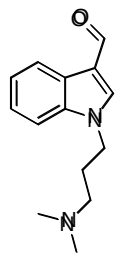
General Procedure 5: To a stirred solution of 2-methyl-1H-indole-3-carbaldehyde (1.75 g, 11.00 mmol), Cs_2CO_3 (11.0 g, 33.0 mmol), and EtOH (20 mL) was added 3-chloro-*N*,*N*-dimethylpropan-1-amine hydrochloride (1.70 g, 11.00 mmol). The resulting solution was heated at reflux for 16 h prior to being quenched with water, extracted with DCM (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude reaction mixture was subjected to flash silica chromatography (95:5 DCM:MeOH) to afford the title compound as a yellow oil (76%); ^{1}H NMR ($CDCl_3$): 10.08 (s, 1H), 8.10 (d, *J* = 6.6 Hz, 1H), 7.5 (d, *J* = 7.2 Hz, 1H), 7.20 (m, 2H), 4.2 (t, *J* = 7.2 Hz, 2H), 2.70 (s, 3H), 2.19 (t, *J* = 6.6 Hz, 2H), 2.12 (s, 6H), 1.83 (quin, *J* = 6.9 Hz, 2H); ^{13}C NMR ($CDCl_3$): 184.1, 148.7, 136.1, 125.1, 122.6, 122.1, 120.0, 113.4, 110.2, 55.6, 44.9, 40.6, 26.8, 9.72; HRMS (m/z): $[M]^+$ calcd. for $C_{15}H_{20}N_2O$, 244.1576; found, 244.1577.

1-(2-Dimethylaminoethyl)-2-methyl-1H-indole-3-carbaldehyde (*Precursor for compound 13b)*



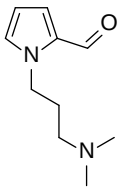
This compound was synthesised utilising general procedure 5 which afforded the title compound as a yellow oil (78%); m.p 50-52ºC. ^{1}H NMR ($CDCl_3$): 10.25 (s, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.24 (m, 2H), 4.29 (t, *J* = 7.2 Hz, 2H), 2.72 (s, 3H), 2.62 (t, *J* = 7.2 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR ($CDCl_3$): 184.4, 148.6, 136.0, 125.2, 122.4, 121.9, 119.9, 113.1 108.9, 55.7, 43.9, 40.4, 8.38; HRMS (m/z): $[M]^+$ calcd. for $C_{14}H_{18}N_2O$, 230.1419; found, 230.1422.

1-(3-dimethylaminopropyl)-1*H*-indole-3-carbaldehyde (*Precursor for compound 17 - 19*)



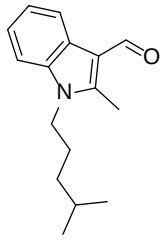
This compound was synthesised utilising general procedure 5, indole-3-carbaldehyde (4.34 g, 30 mmol) and 3-dimethylaminopropylchloride hydrochloride (4.83 g, 30 mmol). The resulting brown solid was purified by flash chromatography (1:9 MeOH/DCM) to afford 1-(3-dimethylaminopropyl)-1*H*-indole-3-carbaldehyde (2.9 g, 42%) as an orange oil. ^{1}H NMR (300 MHz) ($CDCl_3$) δ 9.70 (1 H, s), 8.13 (1 H, dd, *J* = 4.9, 3.1 Hz), 7.50 (1 H, s), 7.15 (1 H, td, *J* = 8.8, 3.4 Hz), 7.11-7.05 (2 H, m), 3.91 (1 2, t, *J* = 6.6 Hz), 1.94 (6 H, s), 1.91 (t, *J* = 7.0 Hz, 2H), 1.68 (2 H, q, *J* = 6.9 Hz); ^{13}C NMR (300 MHz) ($CDCl_3$) δ 183.9, 139.0, 136.6, 124.6, 123.2, 122.1, 121.2, 117.1, 109.7, 55.0, 44.5, 43.8, 26.6; MS (ES+) *m/z* 231 (M+1, 100%)

3-(dimethylamino)propyl)-1H-pyrrole-2-carbaldehyde (*Precursor for compound 22*)



Synthesized utilising general procedure 5 whereby a stirred solution of pyrrole-2-carbaldehyde (1.05 g, 11.00 mmol), Cs_2CO_3 (11.0 g, 33.0 mmol), and EtOH (20 mL) was added 3-chloro-*N*,*N*-dimethylpropan-1-amine hydrochloride (1.70 g, 11.00 mmol). The resulting solution was heated at reflux for 16 h prior to being quenched with water, extracted with DCM (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude reaction mixture was subjected to flash silica chromatography (95:5 DCM:MeOH) to afford 1-(3-(dimethylamino)propyl)-1*H*-pyrrole-2-carbaldehyde (1.65 g , 83%) as a yellow oil; ^{1}H NMR (300 MHz) ($CDCl_3$) δ 9.28 (1 H, s), 6.76 (1 H, t, *J* = 4.1 Hz), 6.68 (1 H, dd, *J* = 4.0, 1.7 Hz), 5.96 (1 H, dd, *J* = 4.1, 2.5 Hz), 4.11 (2 H, t, *J* = 7.0 Hz), 1.96 (1 H, s), 1.66 (2 H, quin, *J* = 6.9 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 178.2, 131.0, 130.5, 123.4, 108.6, 55.2, 46.1, 44.5, 28.2; MS (ES+) *m/z* 181 (M+1, 100%).

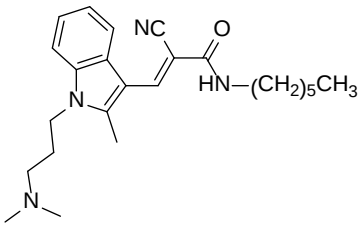
2-methyl-1-(4-methylpentyl)-1H-indole-3-carbaldehyde (*Precursor for compound 20)*



Synthesised using general procedure 5, 3-(dimethylamino)propyl)-1H-pyrrole-2-carbaldehyde, 1, 2-methyl-1H-indole-3-carbaldehyde (3.0 g, 18.9 mmol), 5-bromo-2-methylpentane (3.1 g, 18.9 mmol), to afford the title compound as a yellow oil (2.67 g, 58%). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 10.12 (1 H, s), 8.28 (1 H, td, *J* = 5.7, 2.4 Hz), 7.35-7.19 (3 H, m), 4.00 (2 H, t, *J* = 6.7 Hz), 2.61 (3 H, s), 1.85-1.66 (2 H, m), 1.56 (1 H, sep, *J* = 6.6, Hz) 1.24 (2 H, m), 0.89 (6 H, d, *J* = 6.6 Hz); ^{13}C NMR (300 MHz) ($CDCl_3$) δ 183.6, 146.7, 135.7, 125.3, 122.4, 122.1, 120.3, 113.6, 109.0, 43.1, 35.4, 27.2, 26.9, 21.9, 9.9; MS (ES+) *m/z* 244 (M+1, 100%)

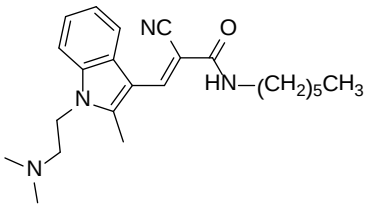
6.3 Preparation of Final Compounds 13a-13c, 19 – 22

2-Cyano-3-[1-(3-dimethylaminopropyl)-2-methyl-1H-indol-3-yl]-N-hexylacrylamide (13a)



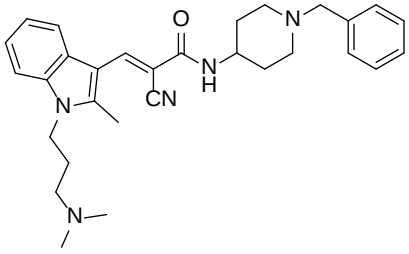
General procedure 6: 1-(2-Dimethylaminoethyl)-2-methyl-1H-indole-3-carbaldehyde (0.2g, 0.87mmol), 2-cyano-*N*-hexylacetamide **(15a)** (0.15g, 0.87 mmol) ethanol (5ml) and piperidine were refluxed for 2hr. After this time water (30 ml) was added to solution. This was then extracted with ethyl acetat (2 x 50 ml). Organic layers were combined, dried over $MgSO_4$ and concentrated under reduced pressure giving an orange solid. This was recrystallized from MeOH giving an orange solid (46%); m.p 100-102 ºC; ^{1}H NMR (DMSO-d_6): 8.35 (s, 1H), 8.10 (br, 1H), 7.98 (d, *J* = 7.1 Hz, 1H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.21 (m, 2H), 4.25 (t, *J* = 6.2 Hz, 2H), 3.22 (q, *J* = 7.1 Hz, 2H), 2.58 (s, 3H) 2.20 (t, *J* = 6.6 Hz, 2H), 2.10 (s, 6H), 1.85 (quin, *J* = 6.9 Hz, 2H) 1.52 (quin, *J* = 7.3 Hz, 2H), 1.27 (m, 6H), 0.89 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6): 164.4, 147.5, 146.3, 138.0, 127.5, 124.1, 123.1, 121.7, 119.5, 112.2, 108.4, 98.6, 56.3, 45.0, 41.5, 32.0, 28.5, 27.8, 26.8, 26.0, 22.9, 14.1, 11.2, 9.7; HRMS (*m/z*): $[M]^+$ calcd. for $C_{24}H_{34}N_4O$, 394.2733; found, 394.2735.

2-Cyano-3-[1(2-dimethylaminoethyl)-2-methyl-1H-indol-3-yl] -N-hexylacrylamide (13b)



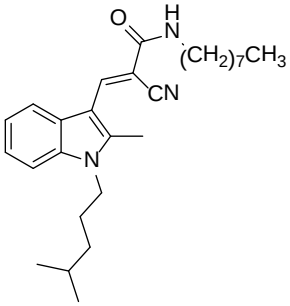
This compound was synthesised utilising general procedure 6 and **15a**. Recrystallization from ethyl acetate afforded to title compound as a yellow solid (67%); m.p 119-121 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): 8.25 (s, 1H), 8.10 (br, 1H), 8.00 (d, *J* = 7.1 Hz, 1H), 7.62 (d, *J* = 7.1 Hz, 1H), 7.20 (m, 2H), 4.32 (t, *J* = 7.2 Hz, 2H), 3.22 (q, *J* = 7.2 Hz, 2H), 2.70 (s, 3H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.30 (s, 6H), 1.46 (quin, *J* = 7.3 Hz, 2H), 1.27 (m, 6H), 0.85 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): 163.1, 145.2, 144.2, 136.2, 125.0, 122.3, 120.9, 19.9, 118.2, 110.4, 106.5, 99.0, 55.8, 44.1, 40.2, 38.7, 28.6, 25.8, 25.2, 21.7, 11.3; HRMS (*m/z*): $[M]^+$ calcd. for $C_{23}H_{32}N_4O$, 380.2576; found, 380.2578.

_N_-(1-benzylpiperidin-4-yl)-2-cyano-3-{1-[3-(dimethylamino)propyl]-2-methyl-1H-indol-3-yl}acrylamide (13c)



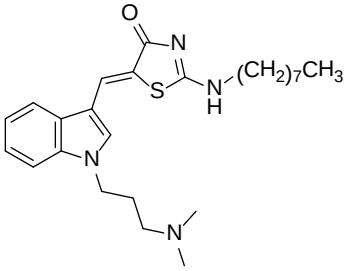
Prepared utilising the general 6 and **15c**, recrystallization from diethyl ether afforded the title compound as a yellow crystalline solid (67%), m.p. 168-169 °C. ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.59 (1 H, s), 8.20-8.10 (1 H, m), 7.43-7.36 (1 H, m), 7.33 (2 H, d, *J* = 4.3 Hz), 7.28 (5 H, m), 6.22 (1 H, d, *J* = 7.8 Hz), 4.23 (2 H, t, *J* = 7.1 Hz), 4.05-3.87 (1 H, m), 3.52 (2 H, s), 2.85 (2 H, m), 2.60 (3 H, s), 2.26 (2 H, t, *J* = 6.7 Hz), 2.23 (6 H, s), 2.01 (2 H, dd, *J* = 9.7, 4.0 Hz), 1.93 (2 H, td, *J* = 13.6, 6.9 Hz), 1.61 (2 H, dq, *J* = 11.6, 3.7 Hz), 1.24 (2 H, t, *J* = 7.0 Hz); ^{13}C NMR (300 MHz) (DMSO-d_6) δ 161.9, 145.3, 144.2, 138.5, 136.7, 128.6, 128.1, 126.8, 124.4, 122.3, 121.1, 121.0, 120.9, 118.3, 110.4, 107.4, 99.3, 62.0, 55.6, 52.1, 47.4, 45.0, 31.2, 26.9, 11.4; MS (ES+) *m/z*: 243.3 (M+2, 100%), 484.3 (M+1, 20%); HRMS calculated for $C_{30}H_{37}N_5O$ Exact Mass: 483.2998; Elemental Analysis: C, 74.50; H, 7.71; N, 14.48; O, 3.31.

2-cyano-3-(2-methyl-1-(4-methylpentyl)-1H-indol-3-yl)-N-octylacrylamide (18)



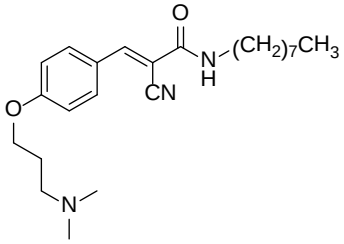
Compound **20** was synthesised using general procedure 6, 2-methyl-1-(4-methylpentyl)-1*H*-indole-3-carbaldehyde (2.30 g, 9.46 mmol), 2-cyano-*N*-octylacetamide (**15b**) (0.45 g, 2.30 mmol), piperidine (0.1 mL) and EtOH (5 mL). Recrystallisation from EtOH afforded the title compound as a yellow solid (2.47 g, 62%), m.p. 96-98 °C; ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.59 (1 H, s), 8.19-8.12 (1 H, m), 7.34-7.23 (3 H, m), 6.37 (1 H, t, *J* = 5.5 Hz), 4.07 (2 H, t, *J* = 7.6 Hz), 3.47 (2 H, q, *J* = 7.6 Hz), 3.46-3.38 (1 H, m), 2.56 (3 H, s), 1.86-1.70 (2 H, m), 1.68-1.57 (2 H, m), 1.68-1.50 (2 H, m), 1.46-1.24 (8 H, m), 1.20 (2 H, t, *J* = 7.6 Hz), 0.89 (9 H, dd, *J* = 6.7, 3.3 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) δ 161.9, 145.5, 144.2, 136.6, 124.9, 122.4, 121.4, 121.2, 119.0, 109.2, 108.7, 96.043.7, 39.9, 35.4, 31.2, 29.0, 28.7, 28.6, 27.2, 26.9, 26.4, 22.0, 21.8, 13.5, 11.3; MS (ES+) *m/z* 422 (M+1, 100%)

5-((1-(3-(dimethylamino)propyl)-1*H*-indol-3-yl)methylene)-2-(octylamino)thiazol-4(5*H*)-one (19)



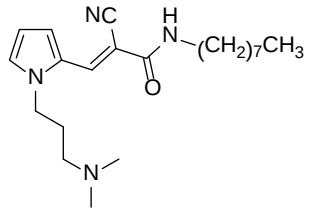
Synthesised utilising general procedure 6, 1-(3-dimethylaminopropyl)-1*H*-indole-3-carbaldehyde (2.45 g, 11.0 mmol), 2-(octylamino)thiazol-4(5H)-one (2.50 g, 11.0 mmol), pipridine (0.1 mL) and EtOH (20 mL). Recrystallization from EtOH afforded the title compound (3.13 g, 65 %) as a yellow solid (mp 136-137 °C);^{1}H NMR (300 MHz) (DMSO-d_6) δ 9.44 (1 H, s), 7.81 (2 H, m), 7.59 (1 H, s), 7.55 (1 H, d, *J* = 8.1 Hz), 7.25 (1 H, t, *J* = 7.3 Hz), 7.17 (1 H, t, *J* = 7.3 Hz), 4.30 (2 H, t, *J* = 6.6 Hz), 3.46 (2 H, t, *J* = 6.6 Hz), 2.15 (8 H, m), 1.96-1.81 (2 H, m), 1.56 (2 H, m), 1.21 (10 H, m), 0.82 (3 H, t, *J* = 5.7 Hz); ^{13}C NMR (75 MHz) (DMSO-d_6) δ 179.8, 172.0, 136.0, 130.1, 129.8, 127.2, 122.7, 120.9, 120.7, 120.3, 118.5, 110.5, 110.3, 55.3, 44.8, 44.2, 43.7, 31.1, 28.5, 28.4, 27.0, 26.2, 22.0, 13.7; MS (ES+) *m/z* 441 (M+1, 100%)

2-cyano-3-(4-(3-(dimethylamino)propoxy)phenyl)-N-octylacrylamide (21)



This compound was synthesised utilising general procedure 6, 2, 4-(3-(dimethylamino)propoxy)benzaldehyde (0.51 g, 2.49 mmol), 2-cyano-*N*-octylacetamide (**15b**) (0.53 g, 2.73 mmol), pipridine (0.1 mL) and EtOH (5 mL). Recrystallisation from EtOH afforded the title compound as a yellow solid (0.69 g, 73%), m.p. 98-100 °C; ^{1}H NMR (300 MHz) ($CDCl_3$) δ 8.23 (1 H, s), 7.91 (2 H, d, *J* = 8.8 Hz), 6.97 (2 H, d, *J* = 8.8 Hz), 6.30 (1 H, t, *J* = 5.2 Hz), 4.10 (2 H, t, *J* = 6.4 Hz), 3.41 (2 H, pent, *J* = 7.08 Hz), 2.50 (2 H, t, *J* = 7.2 Hz), 2.28 (6 H, s), 2.07-1.93 (2 H, m), 1.60 (2 H, m), 1.46-1.10 (10 H, m), 0.88 (3 H, t, *J* = 5.9 Hz);

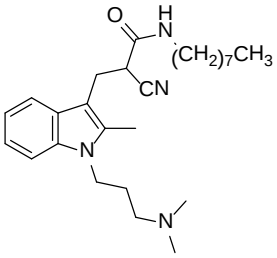
2-cyano-3-[1-(3-(dimethylamino)propyl)-1H-pyrrol-2-yl]-N-octylacrylamide (22)



Compound **22** was synthesised utilising general procedure 6, 1-[3-(dimethylamino)propyl]-1*H*-pyrrole-2-carbaldehyde (0.65 g, 3.6 mmol), 2-cyano-*N*-octylacetamide (**15b**) (0.70 g, 3.6 mmol), pipridine (0.1 mL) and EtOH (5 mL). The resulting solution was quenched with water, extracted with DCM (2 × 50 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude reaction mixture was subjected to flash silica chromatography (95:5 DCM:MeOH) to afford 2-cyano-3-[1-(3-(dimethylamino)propyl)-1H-pyrrol-2-yl]-N-octylacrylamide (0.84 g , 65%) as a yellow oil; ^{1}H NMR (300 MHz) (Acetone-d_6) δ 8.25 (1 H, s), 7.47 (1 H, dd, *J* = 4.0, 1.3 Hz), 7.29 (1 H, s), 7.25 (1 H, dd, *J* = 2.5, 1.6 Hz), 6.35 (1 H, qd, *J* = 4.2, 2.5 Hz), 4.27 (2 H, t, *J* = 6.9 Hz), 3.37 (2 H, q, *J* = 6.5 Hz), 2.23-2.15 (8 H, m), 1.90 (2 H, p, *J* = 6.6 Hz), 1.67-1.52 (2 H, m), 1.30 (10 H, m), 0.86 (3 H, t, *J* = 5.9 Hz); ^{13}C NMR (75 MHz) (Acetone-d_6) δ 160.8, 136.6, 129.1, 126.0, 117.2, 115.9, 110.2, 95.4, 54.8, 43.9, 43.6, 39.4, 31.1, 29.0, 28.7, 28.7, 28.6, 26.2, 21.9, 12.9;
MS (ES+) *m/z* 495 (M+1, 100%)

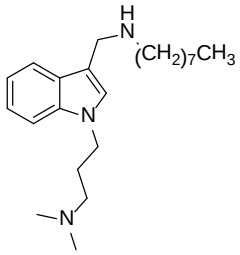
7. Flow Hydrogenation Protocols to Prepare Compounds 14-16

3-amino-2-{[1-(3-dimethylamino)propyl]-2-methyl-1*H*-indol-3-yl}methyl-*N*-octylpropanamide (16)



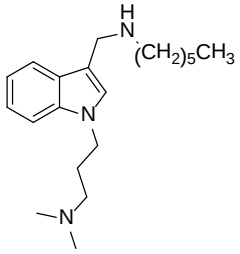
A solution of 2-cyano-3-(1-(3-(dimethylamino)propyl)-2-methyl-1*H*-indol-3-yl)-*N*-octylacrylamide (0.12 g, 0.28 mmol) and EtOH (10 mL) was hydrogenated utilising H-cube system (1.0 ml/min) loaded with a 33mm 10% Pd/C CatCart column heated to 50 °C under 50 bar of H_2 pressure. The eluate was concentrated *in vacuo* to afford 3-amino-2-{[1-(3-dimethylamino)propyl]-2-methyl-1*H*-indol-3-yl}methyl-*N*-octylpropanamide (0.11 g, 92 %) as a yellow oil.^{1}H NMR (300 MHz) ($CDCl_3$) δ 7.50 (1 H, d, *J* = 7.6 Hz), 7.30 (1 H, d, *J* = 8.1 Hz), 7.15 (1 H, t, *J* = 7.4Hz), 7.06 (1 H, t, *J* = 7.4 Hz), 6.13 (1 H, t, *J* = 5.5 Hz), 4.12 (2 H, t, *J* = 7.2 Hz), 3.65 (2 H, td, *J* = 8.1, 6.4 Hz), 3.40 (1 H, ddd, *J* = 22.3, 14.5, 6.8 Hz), 3.22-3.05 (2 H, m), 2.43 (3 H, s), 2.26 (2 H, t, *J* = 6.9 Hz), 2.22 (6 H, s), 1.87 (2 H, p, *J* = 7.0 Hz), 1.40-1.11 (14 H, m), 0.87 (3 H, t, *J* = 6.8 Hz)^{13}C NMR (75 MHz) ($CDCl_3$) δ 164.1, 135.5, 134.4, 126.6, 120.5, 118.7, 118.1, 117.2, 108.62, 104.9, 57.6, 55.96, 44.86, 40.6, 39.9, 39.7, 30.8, 28.4, 27.5, 25.8, 25.5, 21.9, 17.9, 13.5, 9.9;MS (ES+) *m/z* 215 (M+2, 100%), 429 (M+1, 60%)

***N*-(1-(3-dimethylaminopropyl)-1*H*-indol-3-ylmethyl)octylamine (17)**



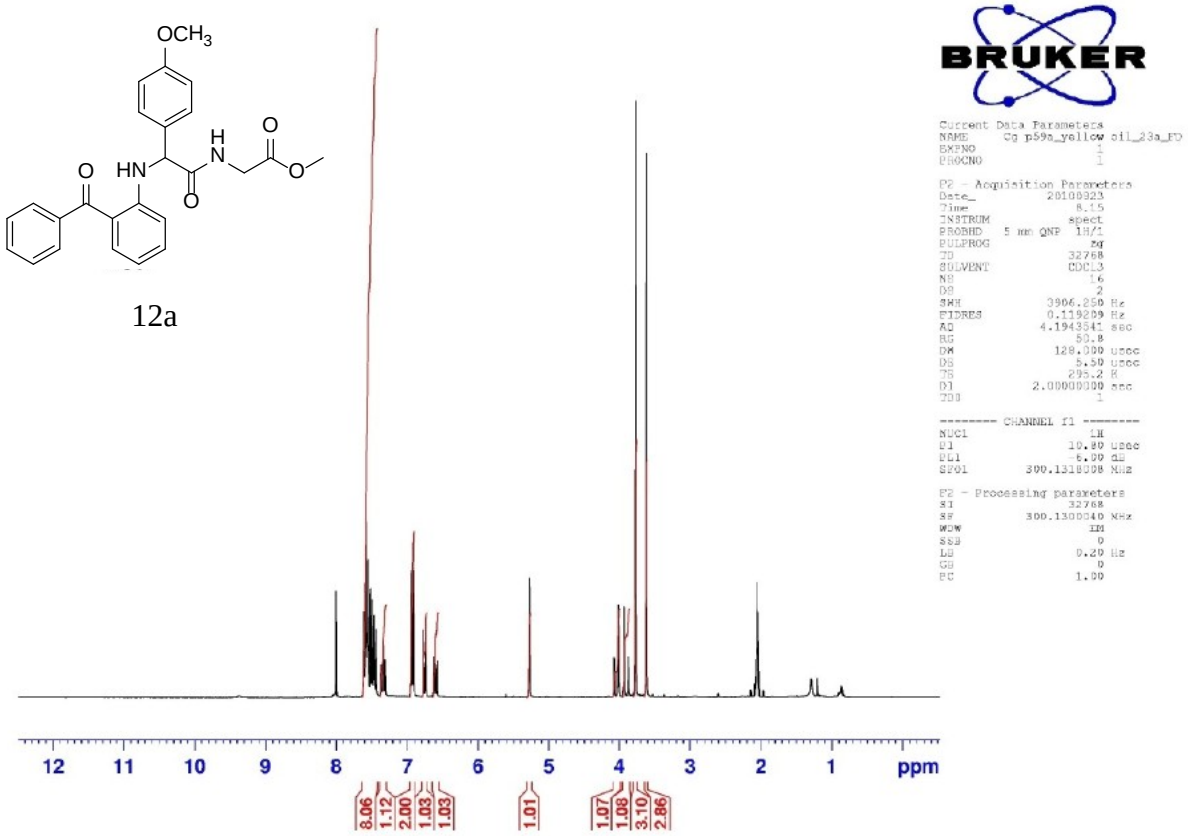
General procedure 7: A solution of 1-(3-dimethylaminopropyl)-1*H*-indole-3-carbaldehyde (0.35 g, 1.52 mmol), hexylamine (0.25 mL, 1.52 mmol), toluene (5.0 mL) and $MgSO_4$ (0.5 g) was irradiated with microwaves (100 °C, 150 W) for 10 mins. The resulting crude material was filtered, diluted with EtOH (30 mL, 0.05 M) and hydrogenated with a H-cube system (1.0 ml/min) loaded with a 33mm 10% Pd/C CatCart column heated to 50 °C under 50 bar. The eluate was concentrated *in vacuo* to afford *N*-(1-(3-dimethylaminopropyl)-1*H*-indol-3-ylmethyl) octylamine (0.43 mg, 78%) as a yellow oil. ^{1}H NMR (300 MHz) ($CDCl_3$) δ 7.62 (1H, d, *J* = 7.8 Hz), 7.34 (1H, d, *J* = 8.1 Hz), 7.21 (1H, m), 7.10 (2H, m), 4.14 (2H, t, *J* = 6.9 Hz), 3.97 (2H, s), 2.68 (2H, t, *J* = 7.2 Hz), 2.23 (2H, t, *J* = 7.2 Hz), 2.20 (6H, s), 1.95 (2H, quin, *J* = 6.9 Hz), 1.53 (2H, quin, *J* = 6.9 Hz), 1.26 (10H, m), 0.86 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) 136.4, 127.5, 126.6, 121.5, 118.9, 118.8, 112.9, 109.5, 56.5, 49.4, 45.4, 44.4, 43.9, 31.8, 29.8, 29.5, 29.2, 28.2, 27.4, 22.6, 14.1; MS (ES+) *m/z* 172 (M+2, 100%), 344 (M+1, 50%)

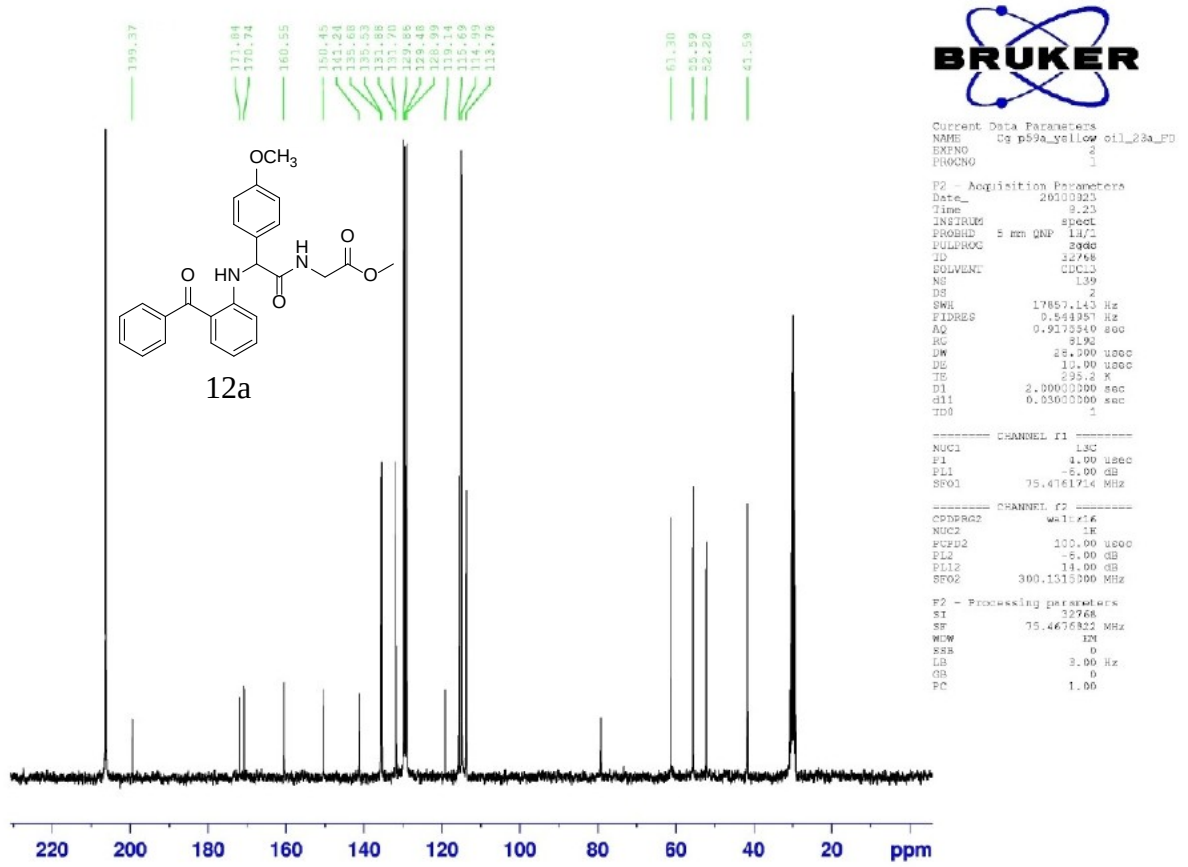
***N*-(1-(3-dimethylaminopropyl)-1*H*-indol-3-ylmethyl)hexylamine (18)**

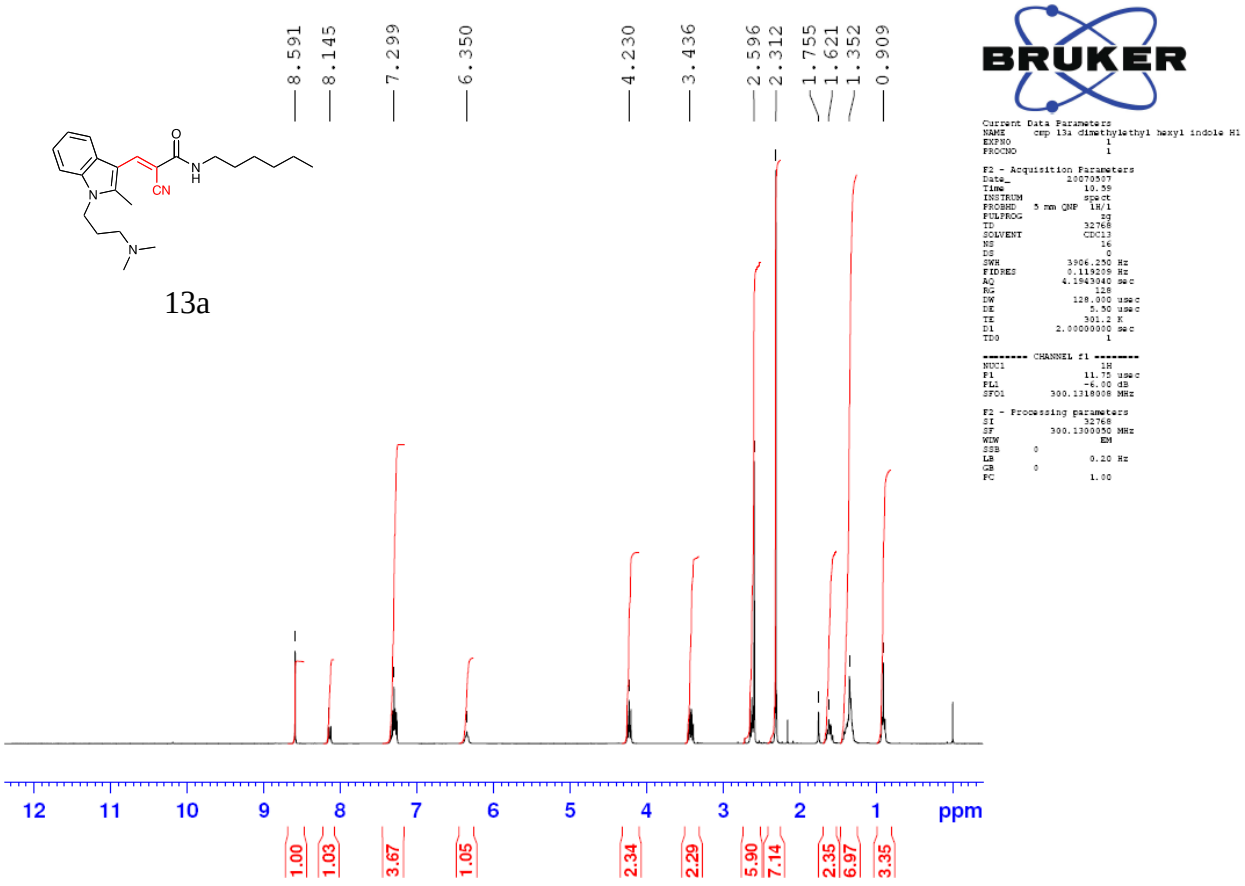


Synthesised utilising general procedure 7, 1-(3-dimethylaminopropyl)-1*H*-indole-3-carbaldehyde (0.38 g, 1.67 mmol) and hexylamine (0.22 mL, 1.67 mmol) affording *N*-(1-(3-dimethylaminopropyl)-1*H*-indol-3-ylmethyl) hexylamine (0.49 g, 93%) as an orange oil. ^{1}H NMR (300 MHz) ($CDCl_3$) δ 7.63 (1H, d, *J* = 7.8 Hz), 7.34 (1H, d, *J* = 8.1 Hz), 7.21 (1H, m), 7.10 (2H, m), 4.14 (2H, t, *J* = 6.9 Hz), 3.98 (2H, s), 2.69 (2H, t, *J* = 7.3 Hz), 2.24 (2H, t, *J* = 7.0 Hz), 2.20 (6H, s), 1.95 (2H, quin, *J* = 6.9 Hz), 1.52 (2H, quin, *J* = 7.1 Hz), 1.30 (6H, m), 0.87 (3H, t, *J* = 6.7 Hz); ^{13}C NMR (75 MHz) ($CDCl_3$) 136.6, 127.7, 126.8, 121.7, 119.1, 119.0, 113.1, 109.7, 56.7, 49.6, 45.6, 44.6, 44.1, 31.9, 29.9, 28.4, 27.2, 22.8, 14.2; MS (ES+) *m/z* 158 (M+2, 100%), 316 (M+1, 70%)

8. NMR of Compounds Displaying Activity Against *Haemonchus contortus*



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4.230
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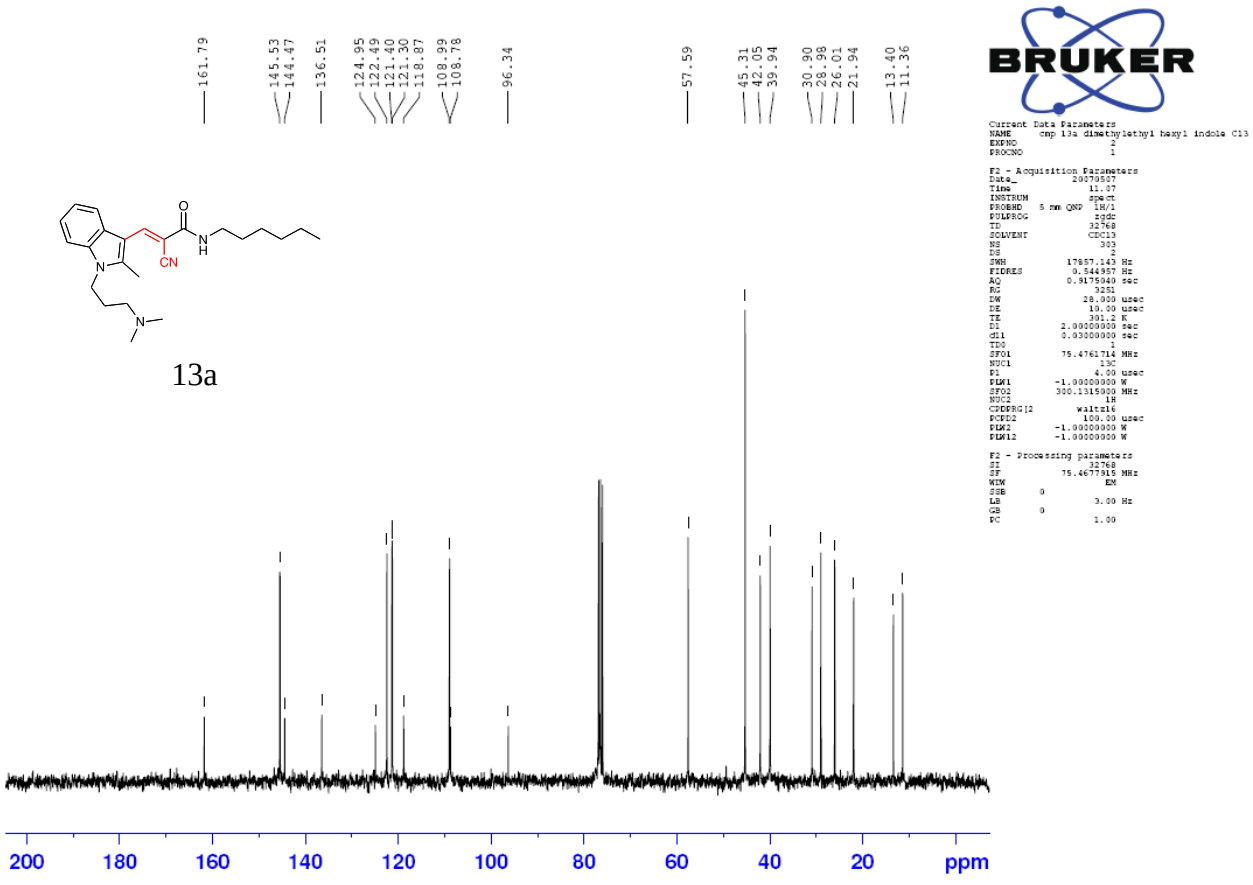
2.312
1.755
1.621
1.352
0.909
CN
13a
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.03
3.67
1.05
2.34
2.29
5.90
7.14
2.35
6.97
3.35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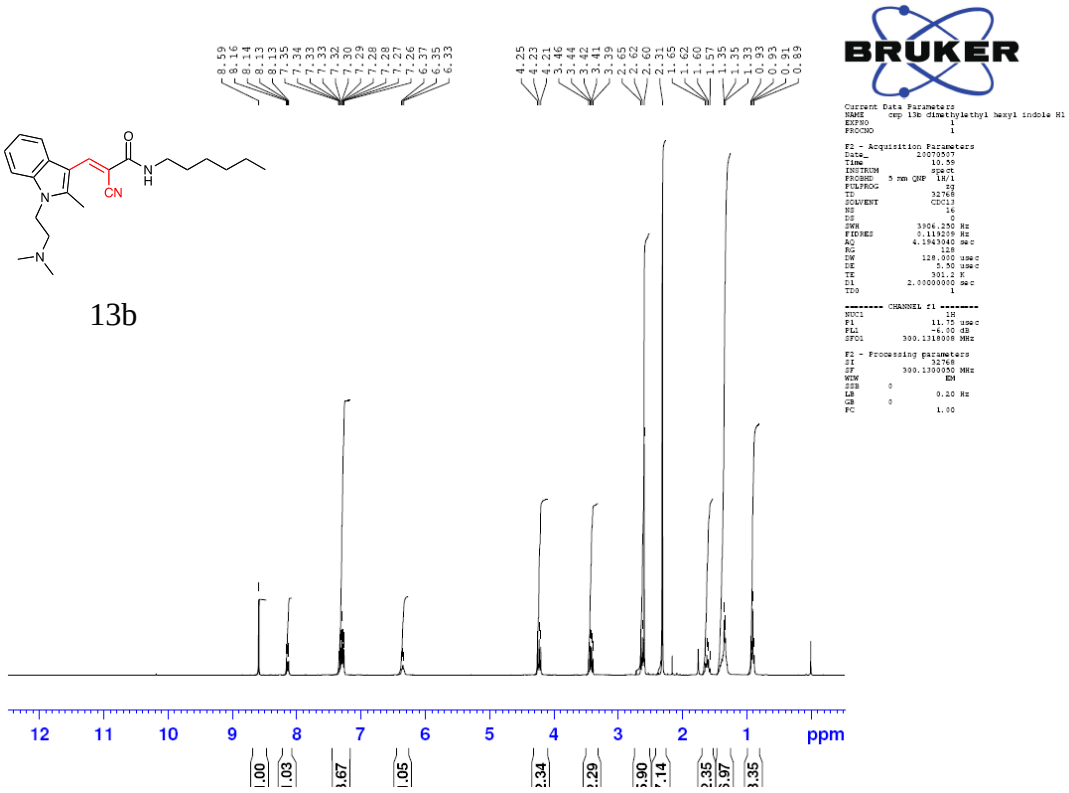
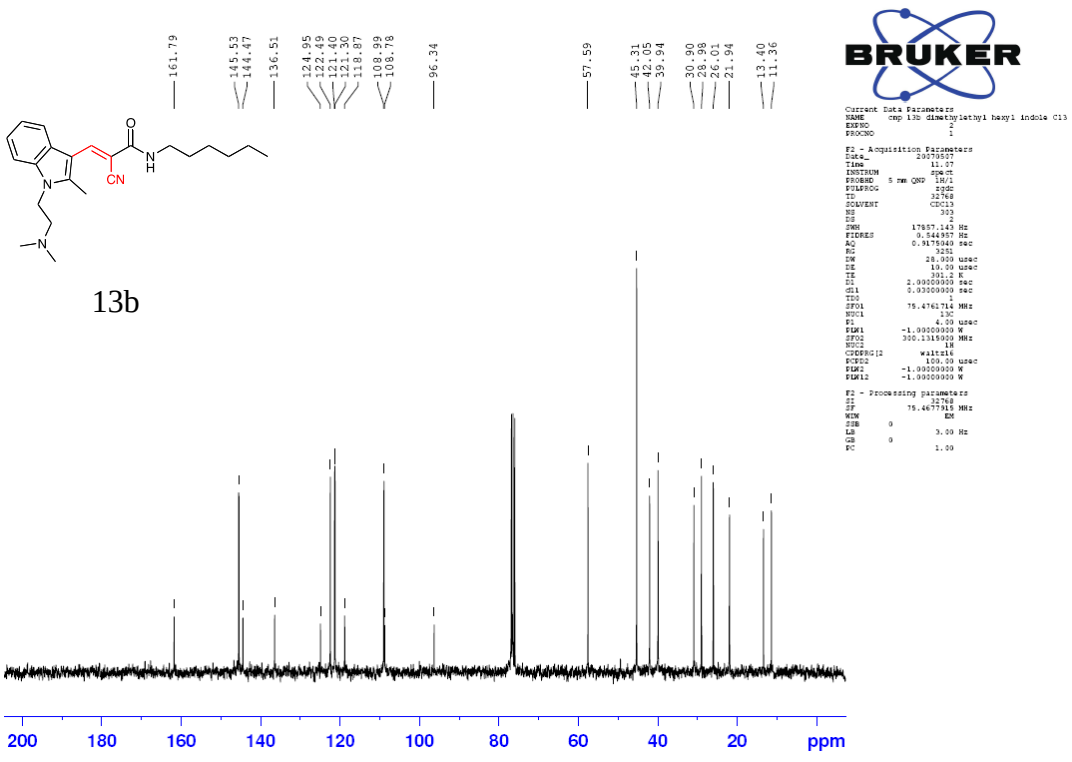
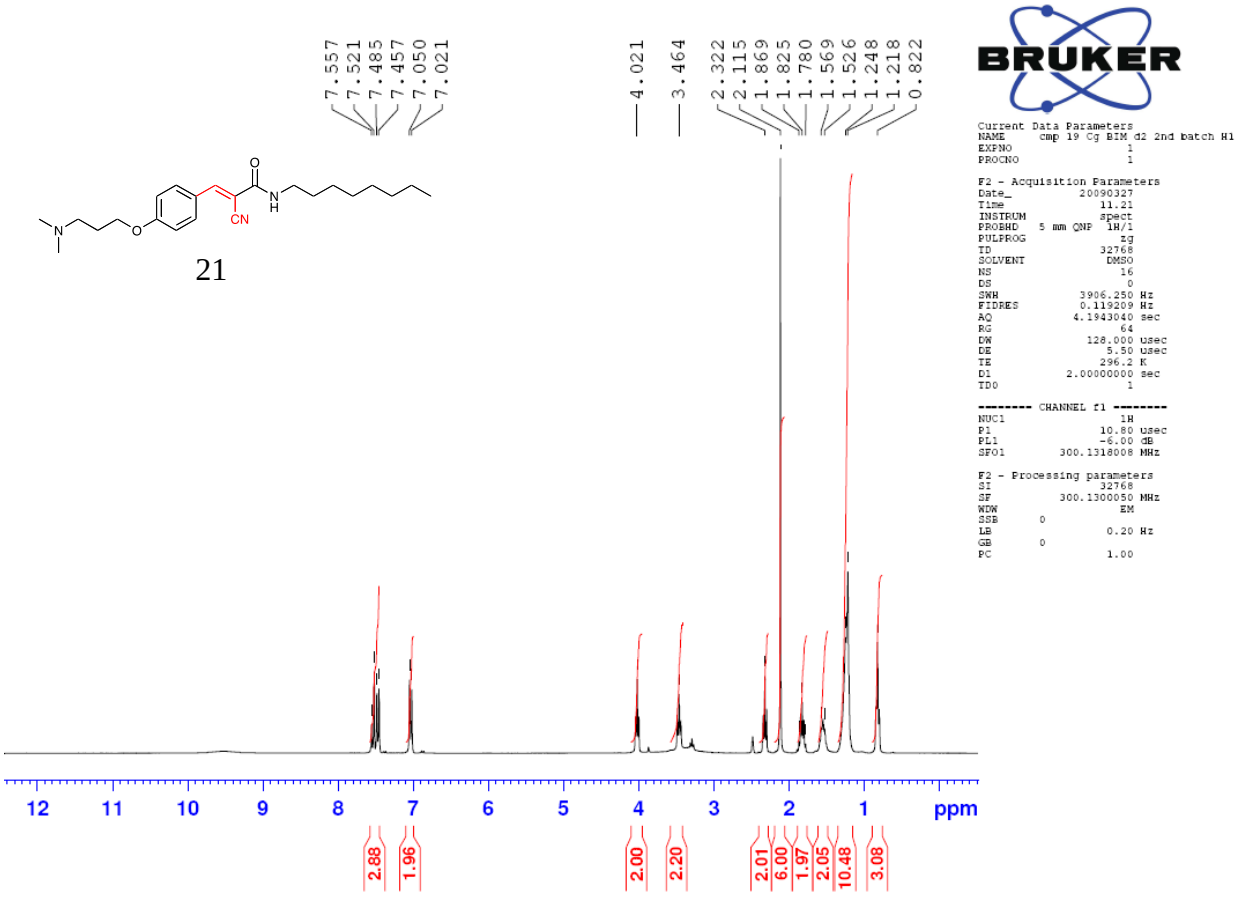
45.31
42.05
39.94
30.90
28.98
26.01
21.94
13.40
11.36
CN
13a
200
180
160
140
120
100
80
60
40
20
ppm


13b
ppm


13b
ppm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7.050
7.021
4.021
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2.322
2.115
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1.825
1.780
1.569
1.526
1.248
1.218
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21
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PROCNO 1
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FIDRES 0.119209 Hz
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DE 5.50 usec
TE 296.2 K
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TD0 1
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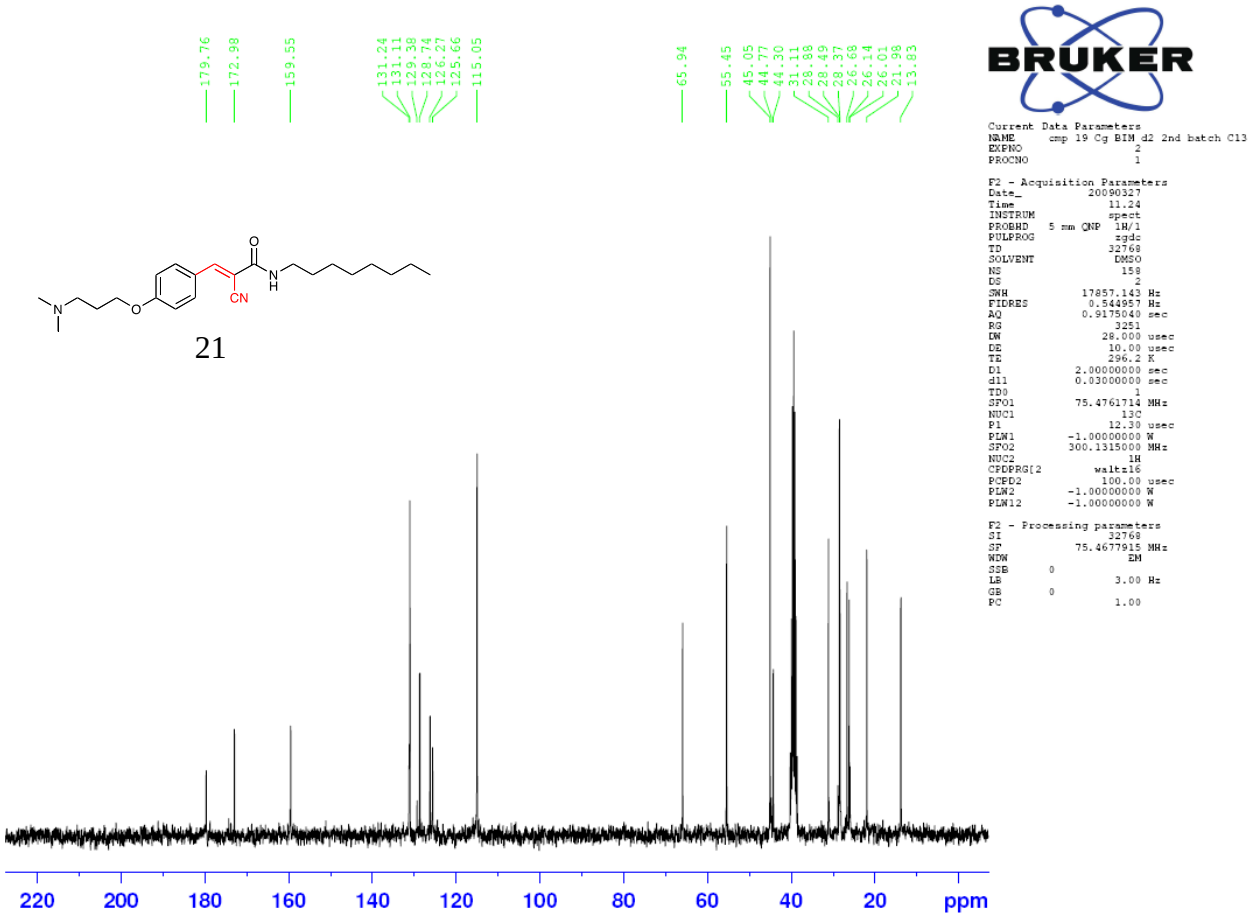
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128.74
126.27
125.66
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65.94
55.45
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44.77
44.30
31.11
28.88
28.49
28.37
26.68
26.14
26.01
21.98
13.83
21
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PROCNO 1
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FIDRES 0.544957 Hz
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DE 10.00 usec
TE 296.2 K
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d11 0.03000000 sec
TD0 1
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NUC1 13C
P1 12.30 usec
PLW1 -1.00000000 W
SFO2 300.1315000 MHz
NUC2 1H
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PCPD2 100.00 usec
PLW2 -1.00000000 W
PLW12 -1.00000000 W
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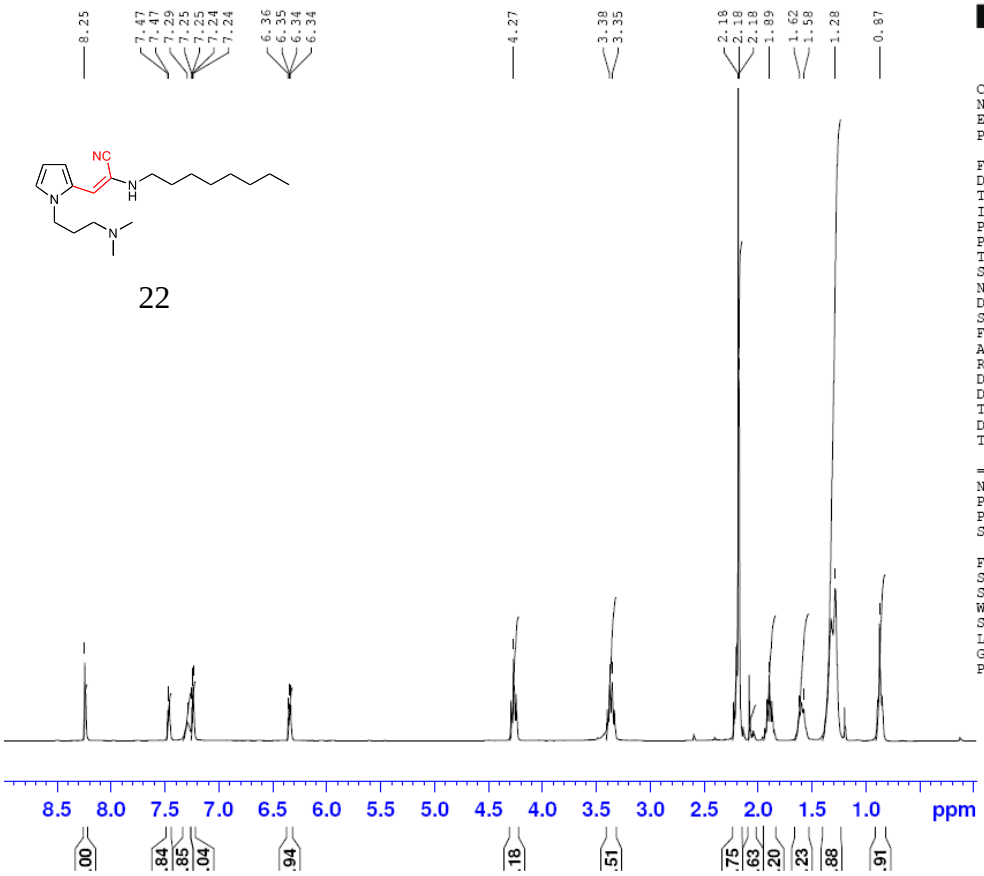

BRUKER

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PL1               -6.00 dB
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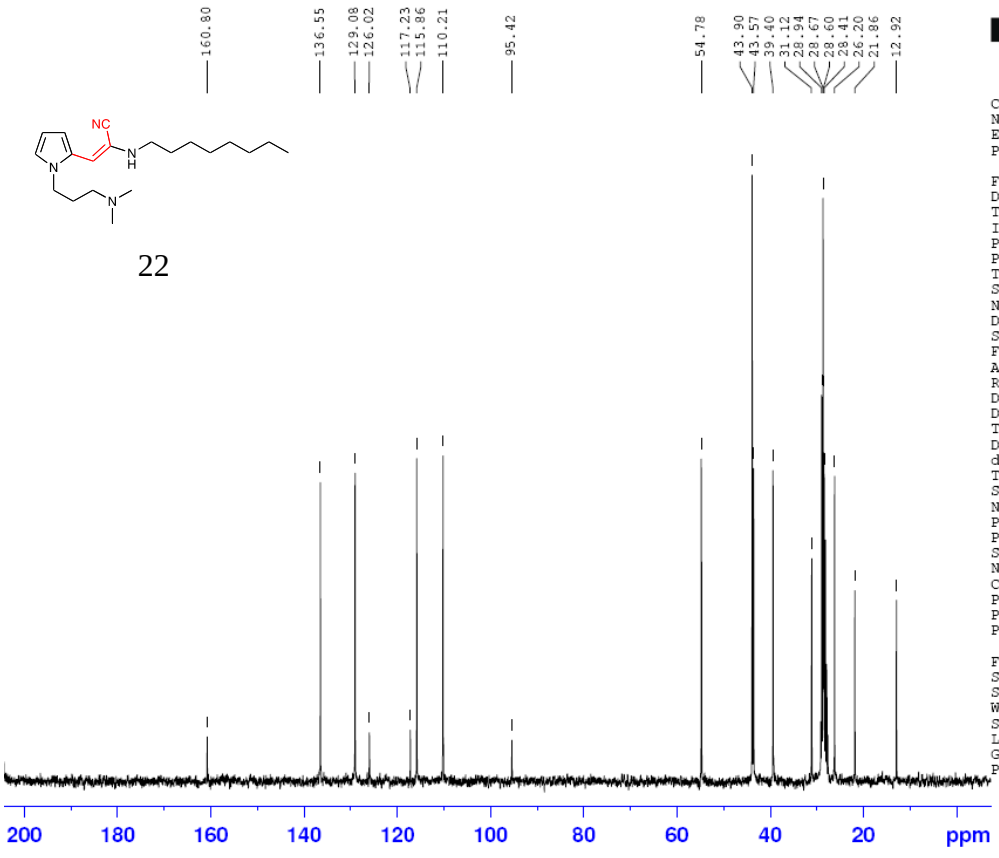

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F2 - Processing parameters
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9. Screening Compounds for Nematocidal Activity

H. contortus (Haecon 5 strain) was raised in helminth-free lambs (Merino crosses; 24 weeks of age), as described by Nikolaou et al.[5] Lambs were infected by intra-ruminal inoculation with 7,000 third-stage larvae (L3s). The patency of infection (21-35 days) was determined by the examination of faeces for the presence of strongylid eggs. Faeces from infected sheep were collected for the immediate isolation of *H. contortus* eggs. Faeces (10 g) were homogenized in 100 ml of sucrose solution (specific gravity: 1.15) and sieved (mesh size: 1 mm). The solution was then placed into a flat dish and transparency film (code PP100C; NOBO) placed on the surface. The sheets were left for 45 min to allow the eggs to stick and then to be removed. The eggs were washed from the sheets with H_20 into a 50 ml centrifuge tube and then diluted further. The tube was then centrifuged at 1,000 x g for 10 min and the sedimented eggs then suspended in 0.5 ml of H_20. Eggs were enumerated by serial dilution, and the number was adjusted to 200 eggs per 20 ml of H_20. The larval development assay (LDA) was conducted as described by Gill et al.[6], with the following modifications. Compounds were tested at 12.5, 25, 50 and 100 mM. Moxidectin® (cydectin, Fort Dodge) was used (at the same concentrations) as a positive control in each experiment. Also MT-CN compounds[1-3] were used as positive controls in each assay, as these compounds had been shown previously to have a toxic effect on *H. contortus.* Aliquots (10 ml) of dilutions of each compound were made in 1.5 ml microcentrifuge tubes, 1 ml of molten agar added, the tube vortexed and the agar aliquotted (150 µl) into the wells of a 96-well microtitre plate. DMSO (1%; i.e. 10 µl of a 100% solution per 1 ml of agar) was used as a solvent-only control in each assay. Two to three replicates were performed for each concentration of each compound. Eggs (n = 200) in 20 µl of water were added to each well and then incubated for 16 h at 27 °C. The number of unhatched eggs in each well was determined, and 15 ml of nutritive medium were then added to each well. Nutritive medium was prepared as follows: 1 g of yeast extract was added to 90 ml of physiological saline and autoclaved for 20 min at 121 °C. Three ml of 10 x Earle's balanced salt solution [EBSS; potassium chloride (KCl) 53 mg/l, sodium bicarbonate ($NaHCO_3$) 261.9 mg/l, sodium chloride (NaCl) 1172.4 mg/l, sodium phosphate monobasic ($NaH_2PO_4.H_2O$) 10.1 mg/ml] were added to 27 ml of yeast extract solution, and the pH adjusted to 5.5 with bicarbonate. Following 6 days of incubation (27 °C), the number of L3s that had developed in each well was counted microscopically at 20-times magnification. Dose-response curves were established for compounds shown to consistently kill *H. contortus* in LDA, by testing concentrations (in 10 µM increments) between 10 and 100 µM. The dose-response assays were repeated at least three times for each compound. The reproducibility of results (i.e. nematocidal activity) for each compound was assessed on different days. The resultant data were expressed as a percentage of mortality against $\log_{10}$ of the concentrations of the compound tested. For each compound, the LD_{50} value (concentration at which 50% of the larvae were killed) was estimated based on the line of best fit to the dose-response curve.

10. Cytotoxicity Evaluation

HT29 (colon), SW480 (colon), MCF-7 (breast), A2780 (ovarian), H460 (lung), A431 (skin), DU145 (prostate), BE2-C (neuronal) and SJ-G2 (brain) cell lines were cultured at 37 ºC under 5% CO_2 in air and were maintained in Dulbecco's modification of Eagle's medium (DMEM; Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/ml), streptomycin (100 mg/ml) and glutamine (4 mM). In a logarithmic phase of growth, cells were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 µl of medium at a density of 2,500-4,000 cells per well. On day 0 (24 h after plating), when the cells were in logarithmic growth, 100 µl of medium with or without the test compound were added to

each well. After 72 h, growth inhibitory effects due to drug exposure were evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, and the absorbance was read at 540 nm. Growth inhibition (%) was determined at a drug concentration of 25 μM. A value of 100% was indicative of total cell growth inhibition. Analogues showing an appreciable percentage of growth inhibition underwent a further dose response analysis, allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is inhibited by 50%, based on the difference between the optical density value on day 0 and that at the end of drug exposure.

11. References

1. A. Ali, M. Bliese, J.-A. M. Rasmussen, R. M. Sargent, S. Saubern, D. G. Sawutz, J. S. Wilkie, D. A. Winkler, K. N. Winzenberg and R. C. J. Woodgate, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 993-997.
2. M. Tarleton, J. Gilbert, M. J. Robertson, A. McCluskey and J. A. Sakoff, *MedChemComm*, 2011, **2**, 31-37.
3. M. Tarleton and A. McCluskey, *Tetrahedron Lett.*, 2011, **52**, 1583-1586.
4. C. P. Gordon, K. A. Young, L. Hizartzidis, F. M. Deane and A. McCluskey, *Org. Biomol. Chem.*, 2011, **9**, 1419-1428.
5. S. Nikolaou, D. Hartman, J. A. Presidente Paul, E. Newton Susan and B. Gasser Robin, *Int J Parasitol*, 2002, **32**, 749-758.
6. J. H. Gill, J. M. Redwin, J. A. Van Wyk and E. Lacey, *Int. J. Parasitol.*, 1991, **21**, 771-776.