**Electronic supplementary Information**

# Synthesis of *dispiro*-cycloalkanones, their *in silico* screening and bioevaluation as antituberculosis and mycobacterial $NAD^+$-dependent DNA ligase inhibitors

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**Contents** **Page No.**

**General Chemistry.** Commercially available reagent grade chemicals were used as received. TLC was carried out with E. Merck Kieselgel 60 $F_{254}$, Spots were visualized under UV light, $I_2$ vapors and by spraying with a 20% aq. $KMnO_4$. Column chromatography was performed on silica gel (230–400 mesh, E. Merck). Optical rotation $[\alpha]_D$ were measured at 25°C on a Rudolph Autopol III polarimeter in MeOH. IR spectra were recorded as thin films or in KBr solution with a Perkin–Elmer Spectrum RX-1 (4000–450 $cm^{-1}$) spectrophotometer. The $^1H$ (200 and 300 MHz) and $^{13}C$ NMR (50 MHz) spectra were recorded on a Avance-200 and Brucker DRX-300 NMR spectrometer in $CDCl_3$. Chemical shift values are reported in ppm relative to TMS as internal reference, unless otherwise stated; s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet); *J* in hertz. FAB mass spectra were performed using a mass Spectrometer Jeol SX-102 and ESI mass spectra with Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

**General Method for the preparation of dispiro cycloalkanones (2-19)**

A mixture of α, α′-(*EE*)-*bis*-(benzylidene)-cycloalkanones/methanone (1 eq.), TMSOI (trimethyl sulphoxonium iodide, 2 eq.) and TBAB (tetra butylammonium bromide, 20 mol%) in $CH_2Cl_2$ (5 ml) was stirred magnetically at ambient temperature for 15 minutes. 50% aq. NaOH (5 ml) solution was subsequently added dropwise and the reaction mixture was refluxed at 80˚C, till the disappearance of α, α′ bis substituted cycloalkanones or methanone. After the completion of the reaction, the reaction mixture was cooled to room temperature and excess of dichloromethane was added and the organic layer was extracted. The organic layer was dried over $Na_2SO_4$ and concentrated under reduced pressure to get the crude product. The latter was purified by column

chromatography ($SiO_2$, 230-400 mesh) using gradient of hexane:ethyl acetate (9:1 → 6:4) to give the desired compounds **2-19** in good yield.

**2,6-Bis-(phenyl)-dispiro[2.1.2.3]decan-4-one (2)**

To the stirred solution of 2,6-(*EE*)-*bis*-(benzylidene)-cyclohexanone (0.50g, 1.80 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.80g, 3.64 mmol), TBAB (0.11g, 0.36 mmol) and 50% NaOH (5 ml) was added as described above gave compound **2** as light yellow solid, yield 0.330g, 60%; mp 80-82 °C; $[\alpha]^{25}_D$ = -10.20 (c, 0.01, MeOH); Anal. Calcd. for $C_{22}H_{22}O$: C, 87.38; H, 7.33;. Found: C, 87.34; H, 7.35. IR (KBr): □ 3020, 2938 (CH), 1660 (CO), 1596 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$+$CCl_4$): $\delta_H$ 7.46-7.20 (m, 10H, ArH), 2.93-2.83 (m, 2H, H-1' and H-1''), 1.97 (dd, *J* = 4.08 and 4.89 Hz, 1H, $H_a$-2'), 1.87 (dd, *J* = 3.93 and 5.07 Hz, 1H, $H_a$-2''), 1.69-1.41 (m, 6H, $CH_2$), 1.30-1.24 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): $\delta_C$ 200.7, 137.4, 136.3, 135.7, 130.8, 129.7, 128.9, 128.5, 127.1, 37.2, 35.7, 29.6, 28.7, 23.3, 22.9, 21.4. MS (ESMS): *m*/*z* 303 $[M+H]^+$.

**2,6-Bis-(4-fluorophenyl)-dispiro[2.1.2.3]decan-4-one (3)**

To the stirred solution of 2,6-(*EE*)-*bis*-(4-fluorobenzylidene)-cyclohexanone (0.50g, 1.61 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.70g, 3.22 mmol), TBAB (0.10g, 0.32 mmol) and 50% NaOH (5 ml) was added as described above gave compound **3** as pale yellow solid, yield 0.354g, 65 %; mp 105-107 °C; $[\alpha]^{25}_D$ = -23.19 (c, 0.01, $CHCl_3$); Anal. Calcd. for $C_{22}H_{20}F_2O$: C, 78.09; H, 5.96;. Found: C, 78.04; H, 5.91. IR (KBr) □ 1657 (CO), 1511 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): $\delta_H$ 7.18-7.11 (m, 4H, ArH), 7.04-6.93 (m, 4H, ArH), 2.88-2.73 (m, 2H, H-1' and H-1'), 1.91-1.77 (m, 2H, $CH_2$), 1.68-1.26 (m, 6H, $CH_2$, $H_a$-2' and $H_a$-2''), 1.17-1.07 (m, 2H, $H_b$-

2' and $H_b$-2''); $^{13}$C NMR (50 MHz, $CDCl_3$ + $CCl_4$): $\delta_C$ 210.4, 164.6, 159.7, 133.0, 131.1, 130.1, 115.7, 115.3, 35.9, 35.1, 34.9, 34.7, 29.0, 28.7, 23.2, 22.9, 22.3. ESMS *m*/*z* = 339 $[M+H]^+$.

**2,6-Bis-(4-chlorophenyl)-dispiro[2.1.2.3]decan-4-one (4)**

To the stirred solution of 2,6-(*EE*)-*bis*-(4-chlorobenzylidene)-cyclohexanone (0.50g, 1.45 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.64g, 2.91 mmol), TBAB (0.09g, 0.29 mmol) and 50% NaOH (5 ml) was added as described above gave compound **4** as light yellow semi solid, yield 0.378g, 70%; $[\alpha]^{25}_D$ = -36.17 (c, 0.01, $CHCl_3$); Anal. Calcd for $C_{22}H_{20}Cl_2O$: C, 71.17; H, 5.43. Found: C, 71.10; H, 5.46. IR (neat) □ 2927 (CH), 1636 (CO) $cm^{-1}$; $^1$H NMR (200 MHz, $CDCl_3$+$CCl_4$): $\delta_H$ 7.30 (d, *J* = 8.72, 4H, ArH), 7.13 (d, *J* = 8.42, 4H, ArH), 2.80-2.72 (m, 2H, H-1' and H-1''), 1.90 (dd, *J* = 6.27 and 7.20 Hz, 2H, $H_a$-2' and $H_a$-2''), 1.68-1.21 (m, 6H, $CH_2$), 1.15 (dd, *J* = 6.27 and 7.20 Hz, 2H, $H_b$-2' and $H_b$-2''); $^{13}$C NMR (50 MHz, $CDCl_3$+$CCl_4$): $\delta_C$ 210.0, 135.8, 133.1, 130.8, 128.8, 128.7, 35.9, 34.9, 29.0, 28.8,23.2, 22.9, 22.3. ESMS *m*/*z* = 372 $[M+H]^+$.

**2,6-Bis-(4-bromophenyl)-dispiro[2.1.2.3]decan-4-one (5)**

To the stirred solution of 2,6-(*EE*)-*bis*-(4-bromobenzylidene)-cyclohexanone (0.50g, 1.15 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.50g, 2.31 mmol), TBAB (0.07g, 0.23 mmol) and 50% NaOH (5 ml) was added as described above gave compound **5** as pale yellow solid, yield 0.372g, 70%; mp 138-140 °C; $[\alpha]^{25}_D$ = -77.32 (c, 0.01, $CHCl_3$); Anal. Calcd for $C_{22}H_{20}Br_2O$: C, 57.42; H, 4.38. Found: C, 57.39; H, 4.40; IR (KBr) □ 2917, 1662, 1591, $cm^{-1}$; $^1$H NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.52-7.03 (m, 8H, ArH), 2.91 (m, 2H, H-1' and H-1''), 1.94 (dd, *J* = 4.06 and 4.86 Hz, 2H, $H_a$-2' and $H_a$-2''), 1.69-1.39 (m, 6H, $CH_2$), 1.24 (dd, *J* = 4.1 and 3.08 Hz, 2H, $H_b$-2' and $H_b$-2''); $^{13}$C NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 200.0, 136.4, 135.7, 135.2, 135.0, 132.2, 132.0, 131.7, 131.2, 123.3, 121.2, 36.3, 35.7, 29.5, 27.8, 23.4, 22.9. ESMS *m*/*z* = 461 $[M+H]^+$.

**2,6-Bis-(4-methoxyphenyl)-dispiro[2.1.2.3]decan-4-one (6)**

To the stirred solution of 2,6-(*EE*)-*bis*-(4-methoxybenzylidene)-cyclohexanone (0.50g, 1.40 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.65g, 2.90 mmol), TBAB (0.10g, 0.30 m mol) and 50% NaOH (5 ml) was added as described above gave compound **6** as a pale yellow solid, yield 0.368g, 68%; mp 98-100 °C; $[\alpha]^{25}_D$ = -23.04 (c, 0.01, $CHCl_3$); Anal. Calcd for $C_{24}H_{26}O_3$: C, 79.53; H, 7.23. Found: C, 79.49; H, 7.26; IR (KBr) □ 2933, 1659, 1514 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.04-6.98 (m, 4H, ArH), 6.77-6.70 (m, 4H, ArH), 3.71 (s, 6H, -$OCH_3$), 2.80-2.59 (m, 2H, H-1' and H-1''), 1.86-1.18 (m, 8H, $CH_2$, $H_a$-2' and $H_a$-2''), 1.07-0.95 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 210.8, 158.8, 130.6, 129.4, 114.0, 55.4, 36.9, 35.1, 29.1, 28.7, 23.2, 22.9, 21.4. ESMS *m*/*z* = 363 $[M+H]^+$.

**2,6-Bis-(3,4-dimethoxyphenyl)-dispiro[2.1.2.3]decan-4-one (7)**

It was obtained by the reaction of 2,6-(*EE*)-*bis*-(3,4-dimethoxybenzylidene)-cyclohexanone (0.50g, 1.20 mmol) with TMSOI (0.55g, 2.53 mmol) and 50% NaOH (5 ml) using TBAB (0.08g, 0.20 mmol ) as described above gave compound 7 as a dark yellow solid, yield 0.374g, 70%; mp 116-118 °C; $[\alpha]^{25}_D$ = -11.74 (c, 0.01, MeOH); Anal. Calcd for $C_{26}H_{30}O_5$: C, 73.91; H, 7.16. Found: C, 73.89; H, 7.21; IR (KBr) □ 2936, 1659, 1515 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 6.81-6.69 (m, 6H, ArH), 3.88 (s, 12H, -$OCH_3$), 2.84, 2.74 (t, *J* = 7.56 Hz, 2H, H-1' and H-1''), 1.93 (dd, *J* = 4.05 and 4.95 Hz, 1H, $H_a$-2'), 1.83 (dd, *J* = 3.87 and 5.13 Hz, 1H, $H_a$-2''), 1.69-1.34 (m, 6H, $CH_2$), 1.18 (dd, *J* = 3.93 and 3.09 Hz, 1H, $H_b$-2'), 1.11 (dd, *J* = 4.11 and 2.91 Hz, 1H, $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 209.3, 147.4, 146.8, 128.2, 120.0, 111.6, 109.5, 54.6, 35.3, 33.7, 27.4, 27.0, 21.7, 21.4, 20.1. ESMS *m*/*z* = 423 $[M+H]^+$.

**2,6-Bis-(3,4,5-trimethoxy phenyl)-dispiro[2.1.2.3]decan-4-one (8)**

It was obtained by the reaction of 2,6-(*EE*)-*bis*-(3,4,5-trimethoxybenzylidene)-cyclohexanone (0.50g, 1.1 mmol) with TMSOI (0.48g, 2.0 mmol) in $CH_2Cl_2$ and 50% aq. NaOH (5 ml) solution using TBAB (0.07g, 0.20 mmol) as described above gave compound **8** as a pale yellow solid, yield 0.424g, 80%; mp 107-109 °C; $[\alpha]^{25}_D$ = -6.06 (c, 0.01, MeOH); Anal. Calcd. for $C_{28}H_{34}O_7$: C, 69.69; H, 7.10. Found: C, 69.62; H, 7.15; IR (KBr): □ 2933, 1663, 1510 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$+$CCl_4$): δ 6.40-6.38 (m, 4H, ArH), 3.85-3.82 (s, 18H, –$OCH_3$), 2.82-2.77 (m, 2H, H-1' and H-1''), 1.83-1.79 (m, 2H, $H_a$-2' and $H_a$-2''), 1.63-1.46 (m, 6H, $CH_2$), 1.11-1.08 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 209.9, 153.5, 138.0, 132.8, 107.4, 106.9, 60.9, 56.6, 36.7, 35.2, 29.3, 28.9, 23.5, 22.5. MS (ESMS): *m*/*z* 483 $[M + H]^+$.

**2,6-Bis-(4-benzyloxyphenyl)-dispiro[2.1.2.3]decan-4-one (9)**

To the stirred solution of 2,6-(*EE*)-*bis*-(4-benzyloxybenzylidene)-cyclohexanone (0.50g, 1.0 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.45g, 2.0 mmol), TBAB (0.07g, 0.20 mmol) and 50% NaOH (5 ml) was added as described above gave compound **9** as light yellow solid, yield 0.370g, 70%; mp 165-167 °C; $[\alpha]^{25}_D$ = -99.39 (c, 0.01, $CHCl_3$); Anal. Calcd. for $C_{36}H_{34}O_3$: C, 84.01; H, 6.66. Found: C, 79.98; H, 6.68; IR (KBr): □ 2928, 1659, 1509 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$+$CCl_4$): δ 7.43-7.30 (m, 10H, ArH), 7.13 (d, *J* = 12.8 Hz, 4H, ArH), 6.93-6.86 (m, 4H, ArH), 5.04 (s, 4H, –$OCH_2$), 2.69 (m, 2H, H-1' and H-1''), 1.94 (dd, *J* = 4.05 and 4.97, 2H, $H_a$-2' and $H_a$-2''), 1.67-1.09 (m, 6H, $CH_2$), 1.09-1.04 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 211.0, 158.1, 137.4, 130.6, 129.7, 128.9, 128.3, 127.8, 115.0, 70.4, 36.9, 35.3, 31.2, 28.7, 23.2, 21.5. MS (ESMS): *m*/*z* 515 $[M + H]^+$.

**2,5-Bis-(4-fluorophenyl)-dispiro[2.1.2.2]nonan-4-one (10)**

To the stirred solution of 2,5-(*EE*)-*bis*-(4-fluorobenzylidene)-cyclopentanone (0.50g, 1.68 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.74g, 3.37 mmol), TBAB (0.10g, 0.33 mmol) and 50% NaOH (5 ml) was added as described above gave compound **10** as a pale yellow oil, yield 0.383g, 70%; $[\alpha]^{25}_D$ = -20.4 (c, 0.01, MeOH); Anal. Calcd. for $C_{28}H_{34}O_7$: C, 77.76; H, 5.59. Found: C, 77.71; H, 5.56; IR (neat): □ 2925, 1603, 1512 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.07-6.93 (m, 8H, ArH), 2.66-2.58 (m, 2H, H-1' and H-1''), 1.82-1.54 (m, 6H, $CH_2$, $H_a$-2' and $H_a$-2''), 1.33-1.27 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 206.0, 165.0, 164.2, 133.1, 130.3, 130.1, 115.8, 115.5, 37.8, 33.6, 25.2, 25.0, 21.8, 20.7. MS (ESMS): *m*/*z* 325 $[M + H]^+$.

**2,5-Bis-(4-bromophenyl)-dispiro[2.1.2.2]nonan-4-one (11)**

To the stirred solution of 2,5-(*EE*)-*bis*-(4-bromobenzylidene)-cyclopentanone (0.50g, 1.19 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.52g, 2.39 mmol), TBAB (0.08g, 0.23 mmol) and 50% NaOH (5 ml) was added as described above gave compound **11** as a dark yellow semi solid, yield 0.346g, 65%; $[\alpha]^{25}_D$ = -24.02 (c, 0.01, $CHCl_3$); Anal. Calcd. for $C_{21}H_{18}Br_2O$: C, 56.53; H, 4.07. Found: C, 56.50; H, 4.11; IR (neat): □ 2921, 1627, 1487 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.54-7.32 (m, 8H, ArH), 2.94-2.67 (m, 4H, H-1', H-1'', $H_a$-2' and $H_a$-2''), 1.97-1.71 (m, 4H, $CH_2$), 1.45-1.40 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 205.7, 137.6, 136.5, 134.9, 132.4, 132.2, 131.0, 130.3, 124.0, 121.1, 38.6, 34.9, 33.3, 27.5, 24.6, 21.8, 20.3. MS (ESMS): *m*/*z* 447 $[M + H]^+$.

**2,5-Bis-(4-methoxyphenyl)-dispiro[2.1.2.2]nonan-4-one (12)**

To the stirred solution of 2,5-(*EE*)-*bis*-(4-methoxybenzylidene)-cyclopentanone (0.50g, 1.56 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.68g, 2.13 mmol), TBAB (0.10g, 0.31 mmol) and 50%

NaOH (5 ml) was added as described above gave compound **12** as a light yellow semi solid, yield 0.353g, 65%; $[\alpha]^{25}_{D}$ = -75.11 (c, 0.01, $CHCl_3$); Anal. Calcd. for $C_{23}H_{24}O_3$: C, 79.28; H, 6.94. Found: C, 79.22; H, 7.00; IR (neat): □ 2929, 1612, 1514 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.05-6.98 (m, 4H, ArH), 6.83-6.77 (m, 4H, ArH), 3.77 (s, 6H, –$OCH_3$), 2.69-2.55 (m, 2H, H-1' and H-1''), 1.82-1.67 (m, 4H, $CH_2$, $H_a$-2'and $H_a$-2''), 1.58-1.47 (m, 2H, $CH_2$), 1.35-1.27 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 210.0, 158.8, 129.8, 129.7, 129.6, 129.5, 114.1, 55.5, 38.0, 37.9, 34.1, 33.3, 25.3, 25.0, 21.4, 20.3. MS (ESMS): *m*/*z* 349 $[M + H]^+$.

**2,5-Bis-(3,4-di-methoxyphenyl)-dispiro[2.1.2.2]nonan-4-one (13)**

To the stirred solution of 2,5-(*EE*)-*bis*-(3,4-dimethoxybenzylidene)-cyclopentanone (0.50g, 1.31 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.57g, 2.63 mmol), TBAB (0.84g, 0.26 mmol) and 50% NaOH (5 ml) was added as described above gave compound **13** as light yellow solid, yield 0.348g, 65%, mp 60-62 °C; $[\alpha]^{25}_{D}$ = -38.79 (c, 0.01, MeOH); Anal. Calcd. for $C_{25}H_{28}O_5$: C, 73.51; H, 6.91. Found: C, 73.49; H, 6.98; IR (KBr): □ 2926, 1656, 1517 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 6.79-6.73 (m, 3H, ArH), 6.64-6.59 (m, 3H, ArH), 3.85-3.82 (s, 12H, –$OCH_3$), 2.66-2.54 (m, 2H, H-1' and H-1''), 1.88-1.71 (m, 4H, $CH_2$, $H_a$-2'and $H_a$-2''), 1.64-1.48 (m, 2H, $CH_2$), 1.36-1.24 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 216.4, 149.2, 148.3, 130.0, 120.5, 112.5, 112.3, 111.4, 56.2, 38.2, 34.4, 25.4, 21.4, 20.3. MS (ESMS): *m*/*z* 409 $[M + H]^+$.

**2,5-Bis-(4-benzyloxyphenyl)-dispiro[2.1.2.2]nonan-4-one (14)**

To the stirred solution of 2,5-(*EE*)-*bis*-(4-benzyloxybenzylidene)-cyclopentanone (0.50g, 1.05 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.47g, 2.11 mmol), TBAB (0.07g, 0.21 mmol) and 50% NaOH (5 ml) was added as described above gave compound **14** as a pale yellow solid, yield 0.370g, 70%; mp 98-100 °C; $[\alpha]^{25}_D$ = -77.23 (c, 0.01, $CHCl_3$); Anal. Calcd. for $C_{35}H_{32}O_3$: C, 83.97; H, 6.44. Found: C, 83.94; H, 6.47; IR (KBr): □ 3021, 1662, 1594 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.38-7.28 (m, 10H, ArH), 7.04-6.98 (m, 4H, ArH), 6.94-6.84 (m, 4H, ArH), 5.01 (s, 4H, –$OCH_2$), 2.66-2.56 (m, 2H, H-1' and H-1''), 1.80-1.67 (m, 4H, $CH_2$, $H_a$-2'and $H_a$-2''), 1.59-1.53 (m, 2H, $CH_2$), 1.34-1.25 (m, 2H, $H_b$-2' and $H_b$-2''); 13C NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 206.5, 158.1, 137.4, 129.9, 129.8, 129.3, 128.9, 128.7, 128.5, 128.4, 127.8, 115.5, 70.4, 38.6, 37.9, 34.1, 33.3, 25.3, 25.0, 21.7, 20.4. MS (ESMS): *m*/*z* 501 $[M + H]^+$.

**2,7-Bis-(4-chlorophenyl)-dispiro[2.1.2.4]undecan-4-one (15)**

To the stirred solution of 2,7-(*EE*)-*bis*-(4-chlorobenzylidene)-cycloheptanone (0.50g, 1.40 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.61g, 2.80 mmol), TBAB (0.09g, 0.28 mmol) and 50% NaOH (5 ml) was added as described above gave compound **15** as a pale yellow semi solid, yield 0.377g, 70%; $[\alpha]^{25}_D$ = -11.06 (c, 0.01, MeOH); Anal. Calcd for $C_{23}H_{22}Cl_2O$: C, 71.69; H, 5.75. Found: C, 71.67; H, 5.80; IR (neat) □ 2929, 1662, 1598 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.29-7.23 (m, 4H, ArH), 7.14-7.08 (m, 4H, ArH), 2.86-2.78 (m, 2H, H-1' and H-1''), 1.68-1.52 (m, 4H, $CH_2$, $H_a$-2' and $H_a$-2''), 1.46-1.26 (m, 4H, $CH_2$), 1.21-1.12 (m, 2H, $CH_2$), 0.88-0.83 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 210.5, 136.4, 132.9, 130.8, 130.6, 128.8, 38.5, 38.4, 32.5, 28.1, 27.3, 26.8, 20.7, 20.5. ESMS *m*/*z* = 386 $[M+H]^+$.

**2,7-Bis-(4-methoxyphenyl)-dispiro[2.1.2.4]undecan-4-one (16)**

To the stirred solution of 2,7-(*EE*)-*bis*-(4-methoxybenzylidene)-cycloheptanone (0.50g, 1.43 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.63g, 2.87 mmol), TBAB (0.09g, 0.28 mmol) and 50% NaOH (5 ml) was added as described above gave compound **16** as a pale yellow semi solid, yield 0.351g, 65%; $[\alpha]^{25}_D$ = -29.48 (c, 0.01, MeOH); Anal. Calcd for $C_{25}H_{28}O_3$: C, 79.75; H, 7.50. Found: C, 79.71; H, 7.54; IR (neat)  2931, 1675, 1508 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.32 (d, *J* = 8.74 Hz, 2H, ArH), 7.05 (d, *J* = 8.60 Hz, 2H, ArH), 6.88-6.72 (m, 4H, ArH), 3.74 (s, 6H, -$OCH_3$), 2.86-2.78 (m, 2H, H-1' and H-1''), 2.60-2.55 (m, 2H, $H_a$-2' and $H_a$-2''), 1.76-1.27 (m, 8H, $CH_2$), 1.17-1.12 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 207.6, 159.7, 158.8, 142.7, 133.06, 131.5, 130.4, 129.8, 129.0, 55.5, 37.0, 34.5, 31.2, 28.3, 27.6, 27.5, 26.6, 22.2. ESMS *m*/*z* = 377 $[M+H]^+$.

**2,7-Bis-(3,4-dimethoxyphenyl)-dispiro[2.1.2.4]undecan-4-one (17)**

To the stirred solution of 2,7-(*EE*)-*bis*-(3,4-dimethoxybenzylidene)-cycloheptanone (0.50g, 1.22 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.54g, 2.45 mmol), TBAB (0.08g, 0.24 mmol) and 50% NaOH (5 ml) was added as described above gave compound **17** as a light yellow semi solid, yield 0.363g, 68%; $[\alpha]^{25}_D$ = -16.71 (c, 0.01, MeOH); Anal. Calcd for $C_{27}H_{32}O_5$: C, 74.29; H, 7.39. Found: C, 74.26; H, 7.44; IR (neat)  2934, 1673, 1508 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 6.81-6.68 (m, 6H, ArH), 3.87 (s, 12H, -$OCH_3$), 2.86-2.72 (m, 2H, H-1' and H-1''), 1.66-1.30 (m, 10H, $CH_2$, $H_a$-2' and $H_a$-2''), 1.19-1.14 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 210.9, 148.7, 147.7, 130.1, 120.9, 112.9, 112.8, 111.0, 55.8, 38.0, 32.6, 32.3, 29.6, 27.8, 26.9, 26.4, 20.2. ESMS *m*/*z* = 437 $[M+H]^+$.

**2,7-Bis-(3,4,5-trimethoxyphenyl)-dispiro[2.1.2.4]undecan-4-one (18)**

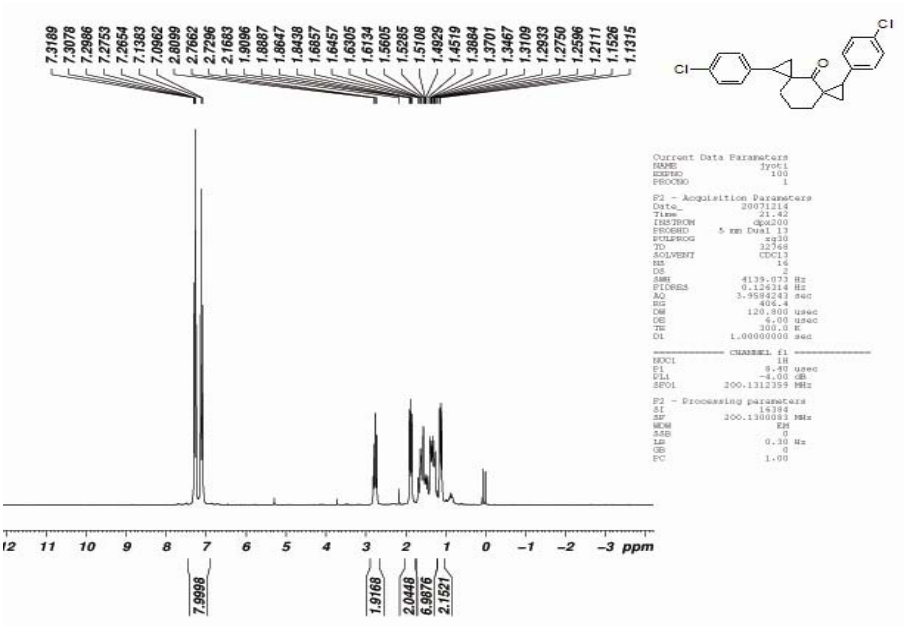
To the stirred solution of 2,7-(*EE*)-*bis*-(3,4,5-trimethoxybenzylidene)-cycloheptanone (0.50g, 1.06 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.47g, 2.13 mmol), TBAB (0.07g, 0.21 mmol) and 50% NaOH (5 ml) was added as described above gave compound **18** as a pale yellow solid, yield 0.370g, 70%; mp 140-142 °C; $[\alpha]^{25}_D$ = -37.87 (c, 0.01, MeOH); Anal. Calcd for $C_{29}H_{36}O_7$: C, 70.14; H, 7.31. Found: C, 70.11; H, 7.35; IR (KBr)  2933, 1662, 1505 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 6.64 (s, 2H, ArH), 6.39 (s, 2H, ArH), 3.86 (s, 12H, -$OCH_3$), 3.00-2.92 (m, 2H, H-1' and H-1''), 2.78-2.56 (m, 2H, $H_a$-2' and $H_a$-2''), 2.00-1.53 (m, 8H, $CH_2$), 1.19-1.14 (m, 2H, $H_b$-2' and $H_b$-2''); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 206.7, 153.4, 143.7, 138.7, 137.6, 133.9, 133.4, 131.7, 107.3, 106.8, 61.1, 56..5, 37.1, 34.8, 28.4, 28.2, 27.7, 27.0, 22.4. ESMS *m*/*z* = 497 $[M+H]^+$.

**Bis-[2-(4-benzyloxyphenyl)-cyclopropyl]-methanone (19)**

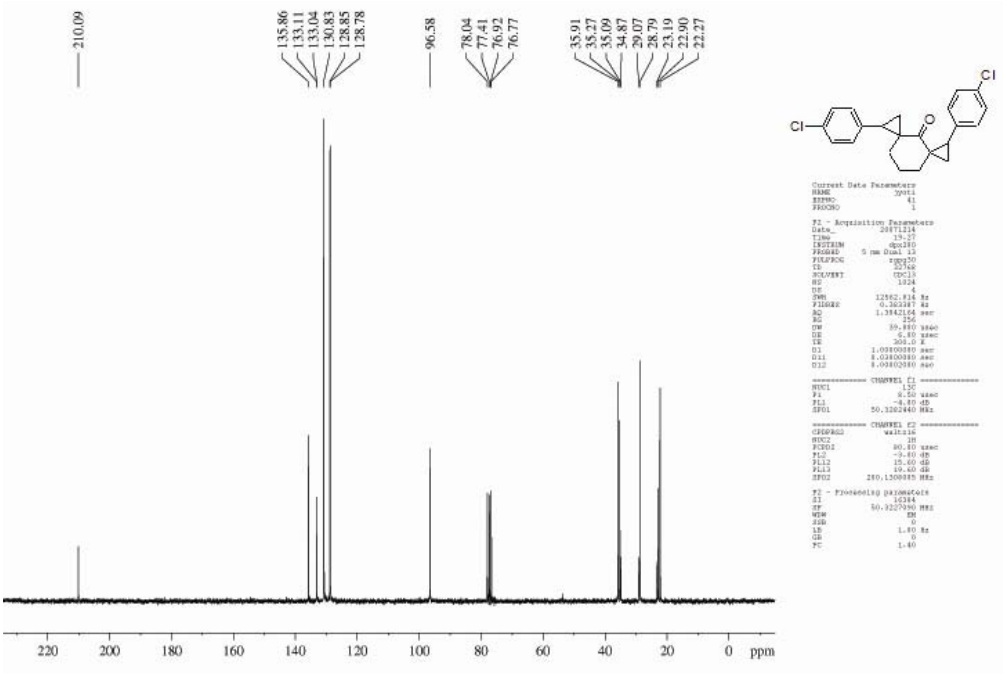
To the stirred solution of α, α′-(*EE*)-*bis*-(4-(benzyloxy benzylidene)-methanone (0.50g, 1.12 mmol) in $CH_2Cl_2$ (5 ml), TMSOI (0.49g, 2.24 mmol), TBAB (0.07g, 0.22 mmol) and 50% NaOH (5 ml) was added as described above gave compound **19** as a white solid, yield 0.345g, 65%; mp 155-157 °C; $[\alpha]^{25}_D$ = +9.66 (c, 0.01, $CHCl_3$); Anal. Calcd for $C_{33}H_{30}O_3$: C, 83.51; H, 6.37. Found: C, 83.56; H, 6.39; IR (KBr)  2891, 1661, 1517 $cm^{-1}$; $^1H$ NMR (200 MHz, $CDCl_3$+$CCl_4$): δ 7.41-7.28 (m, 10H, ArH), 7.03-6.97 (m, 4H, ArH), 6.88-6.84 (m, 4H, ArH), 5.02 (s, 4H, -$OCH_2$), 2.50-2.46 (m, 2H, H-1 and H-7), 2.30-2.26 (m, 2H, H-3 and H-5), 1.72-1.60 (m, 2H, $H_a$-6 and $H_a$-2), 1.35-1.25 (m, 2H, $H_b$-6 and $H_b$-2); $^{13}C$ NMR (50 MHz, $CDCl_3$+$CCl_4$): δ 207.2, 157.9, 137.4, 133.1, 128.9, 128.3, 127.8, 127.6, 115.3, 70.4, 33.5, 33.1, 31.2, 29.2, 28.9, 19.4, 19.2. ESMS *m*/*z* = 475 $[M+H]^+$.

## Copies of 1H and 13C NMR spectra of selected compounds

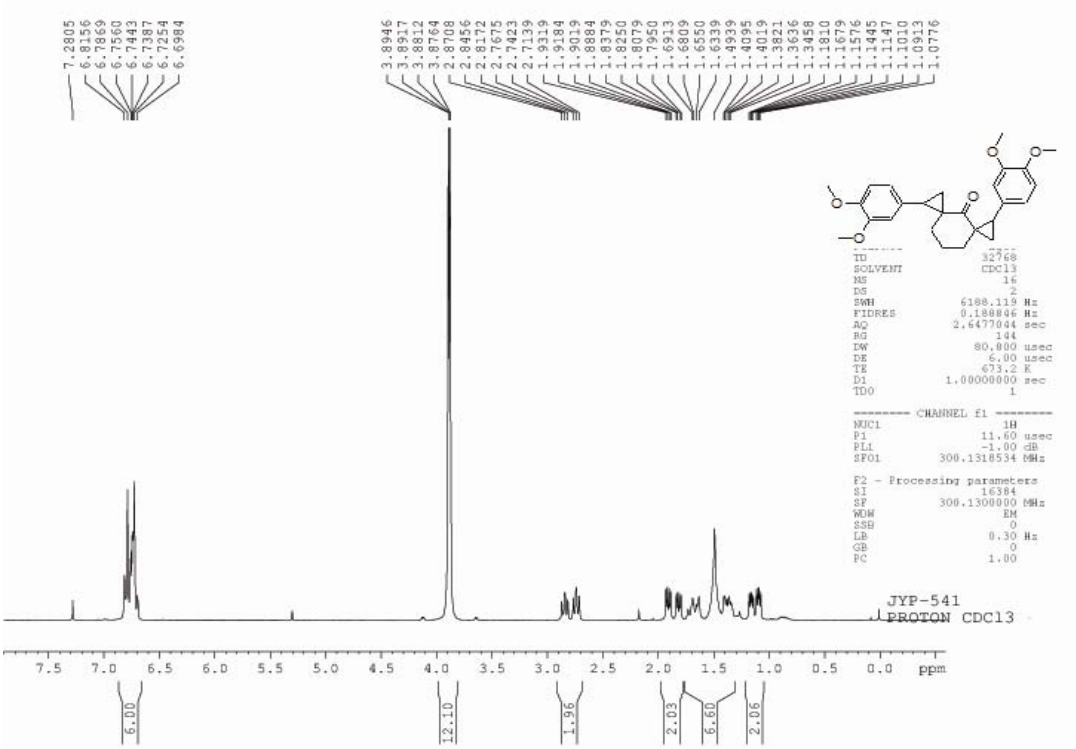
$^{1}$H NMR of compound (**4**)




$^{13}$C NMR of compound (**4**)



## $^{1}$H NMR of compound (**7**)

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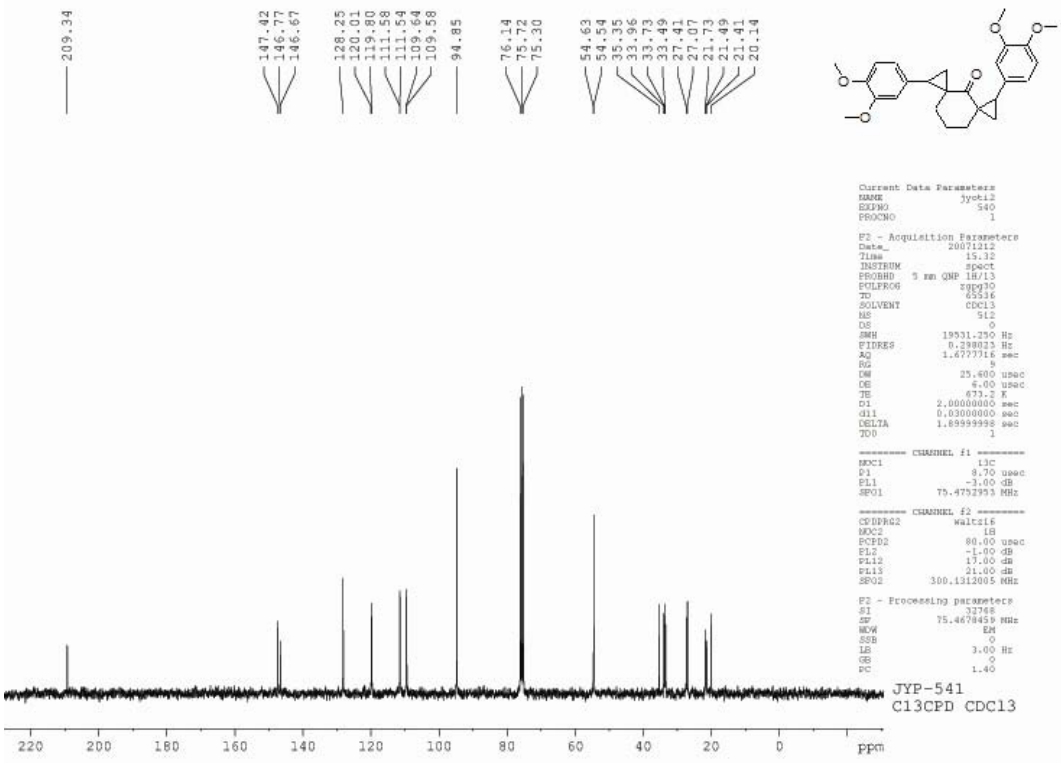
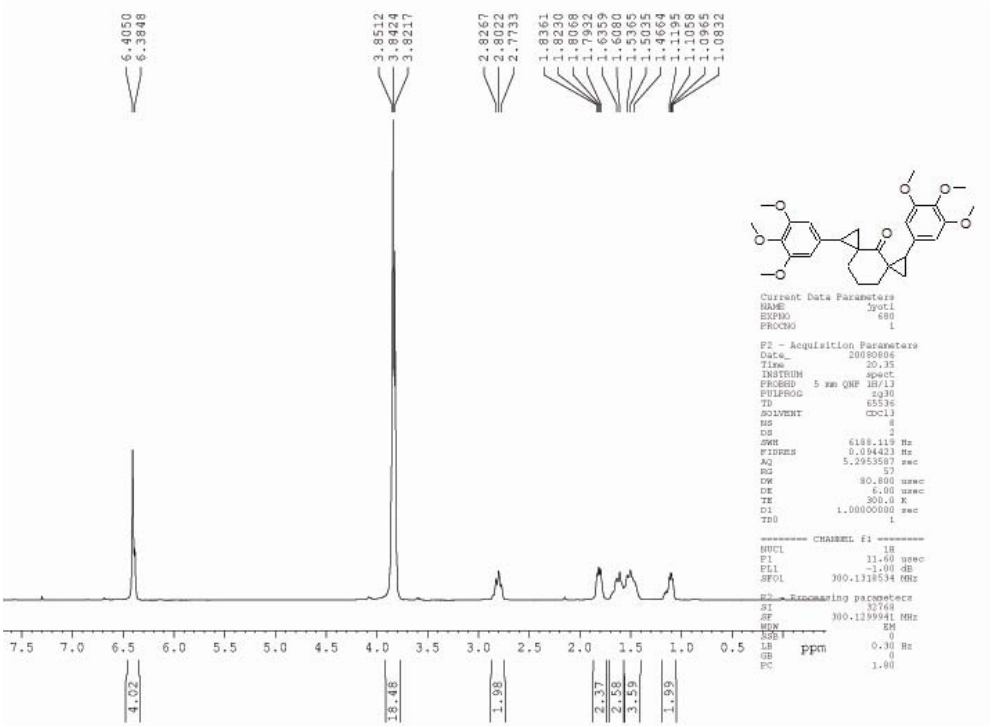


## $^{1}$H NMR of compound (**8**)

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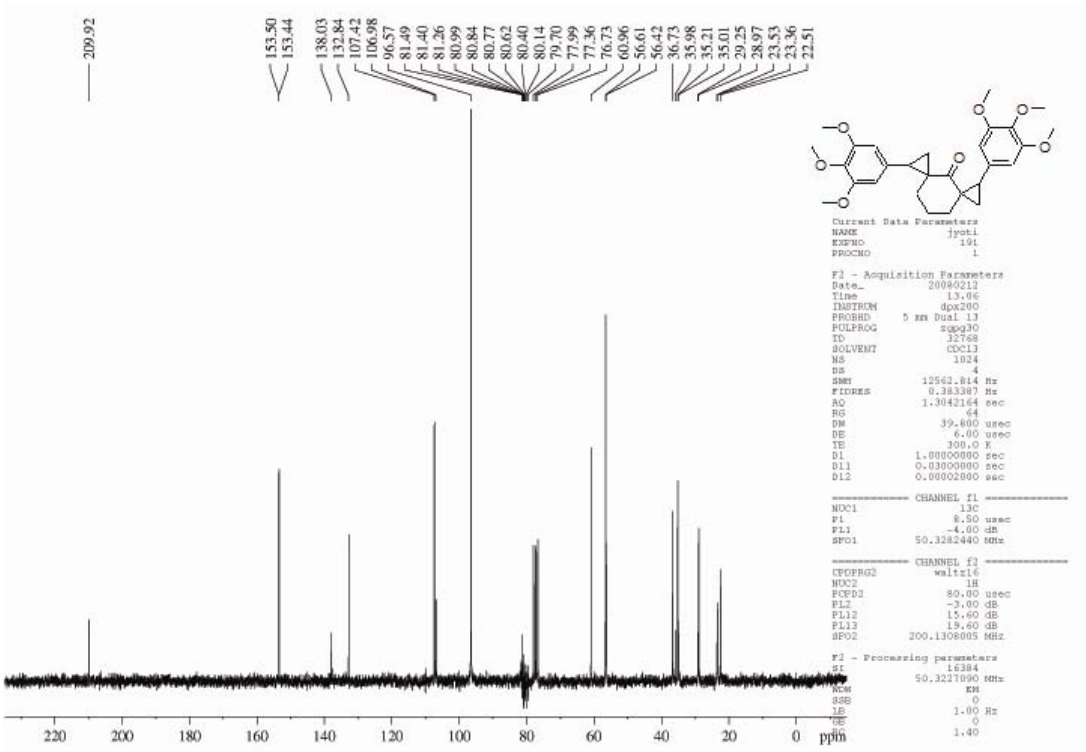
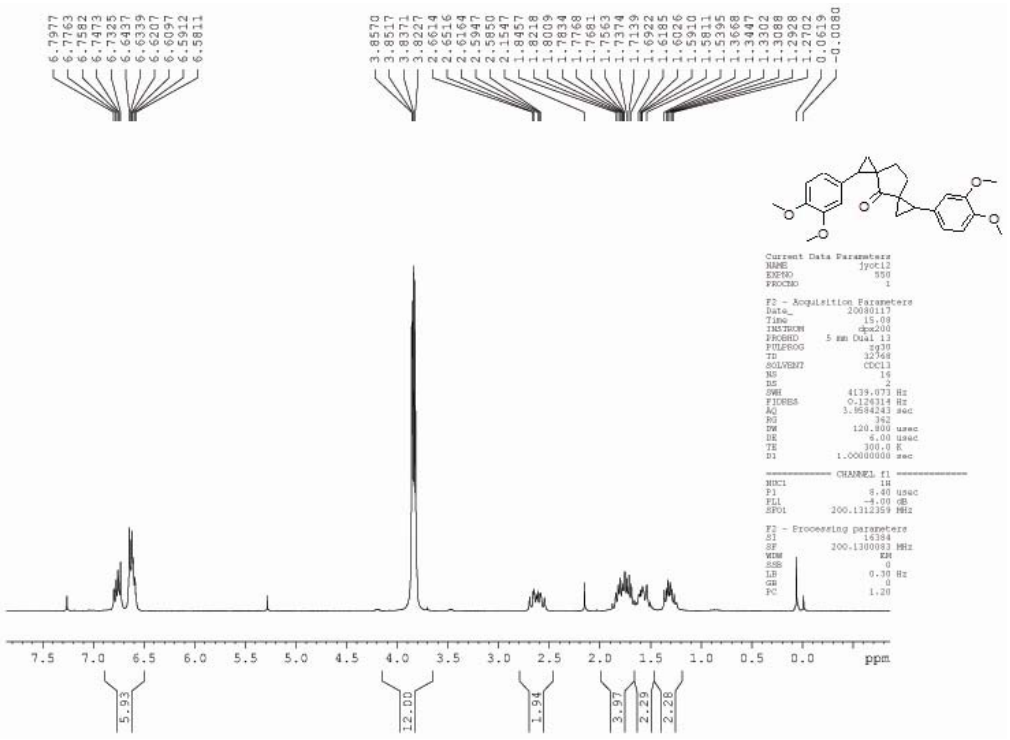


## $^{1}$H NMR of compound (**13**)



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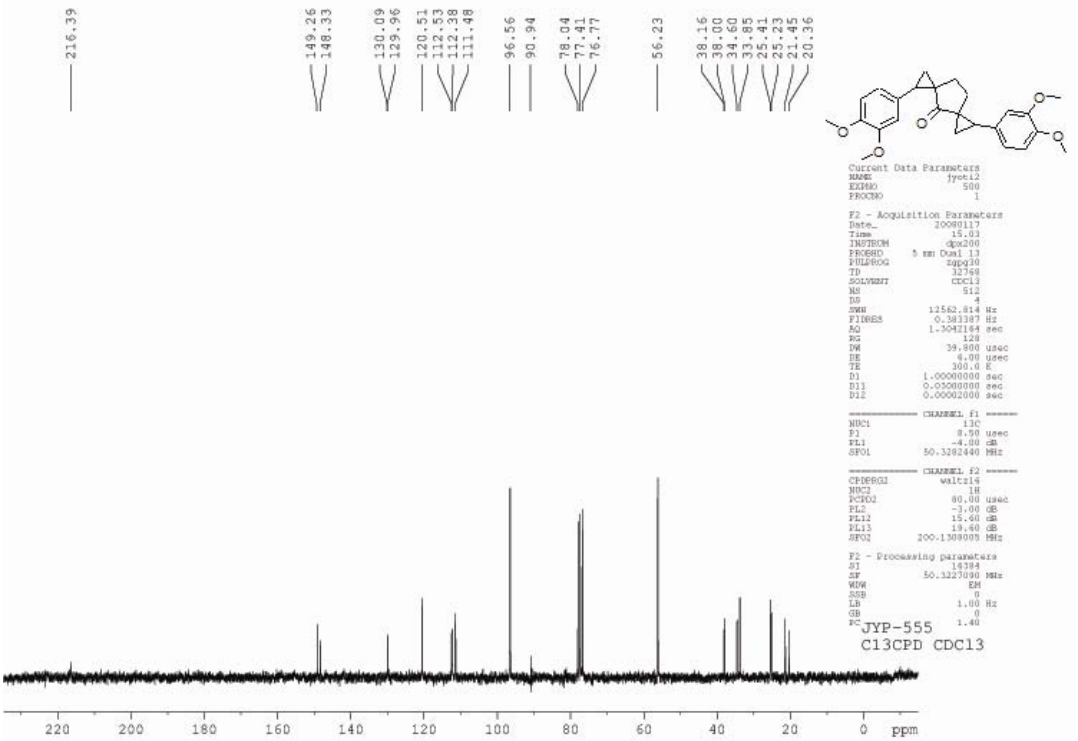

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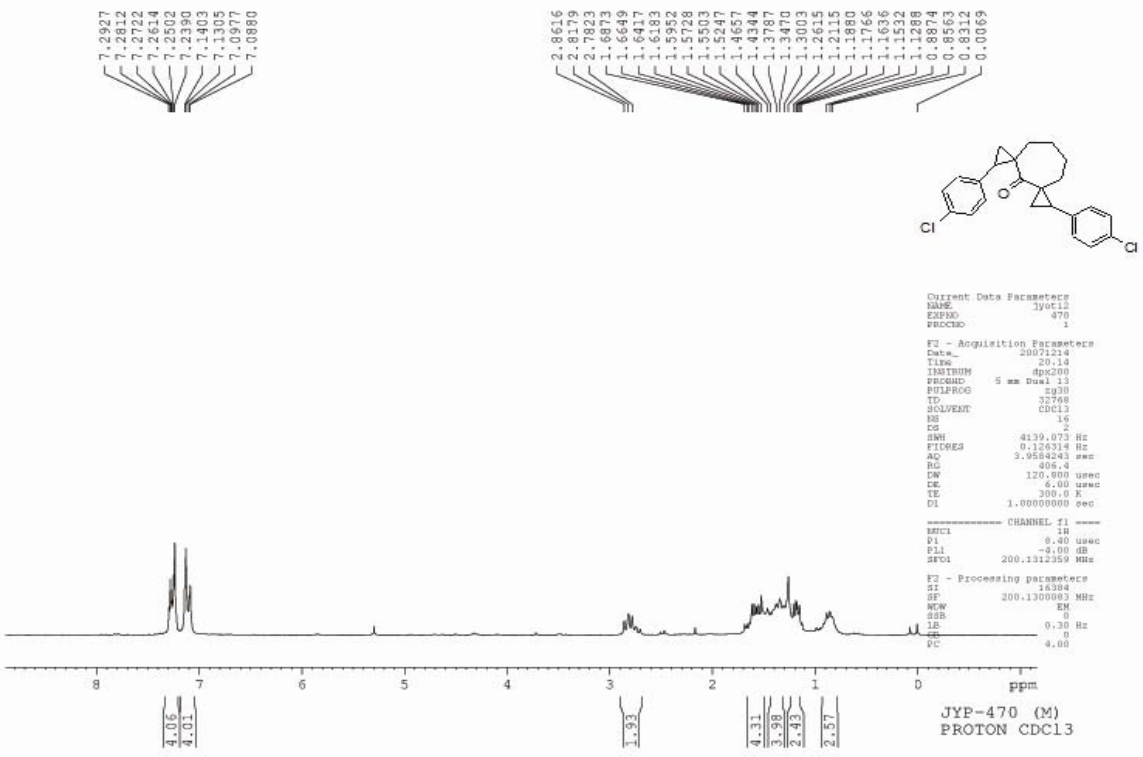


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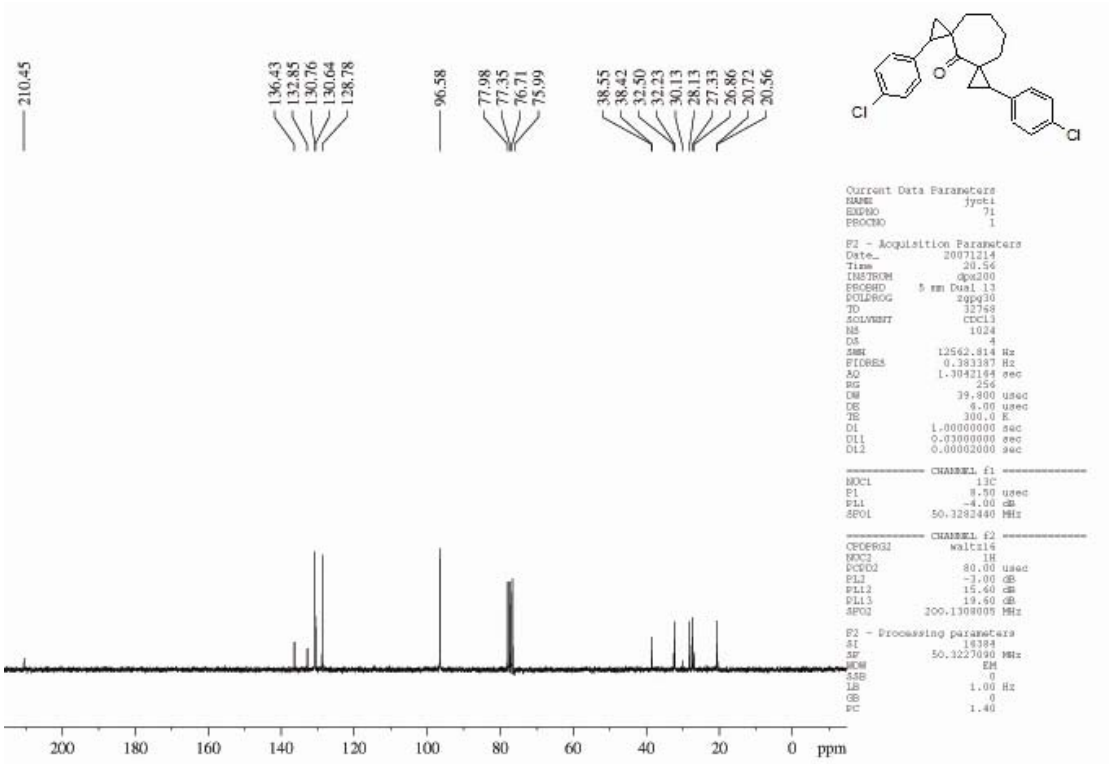
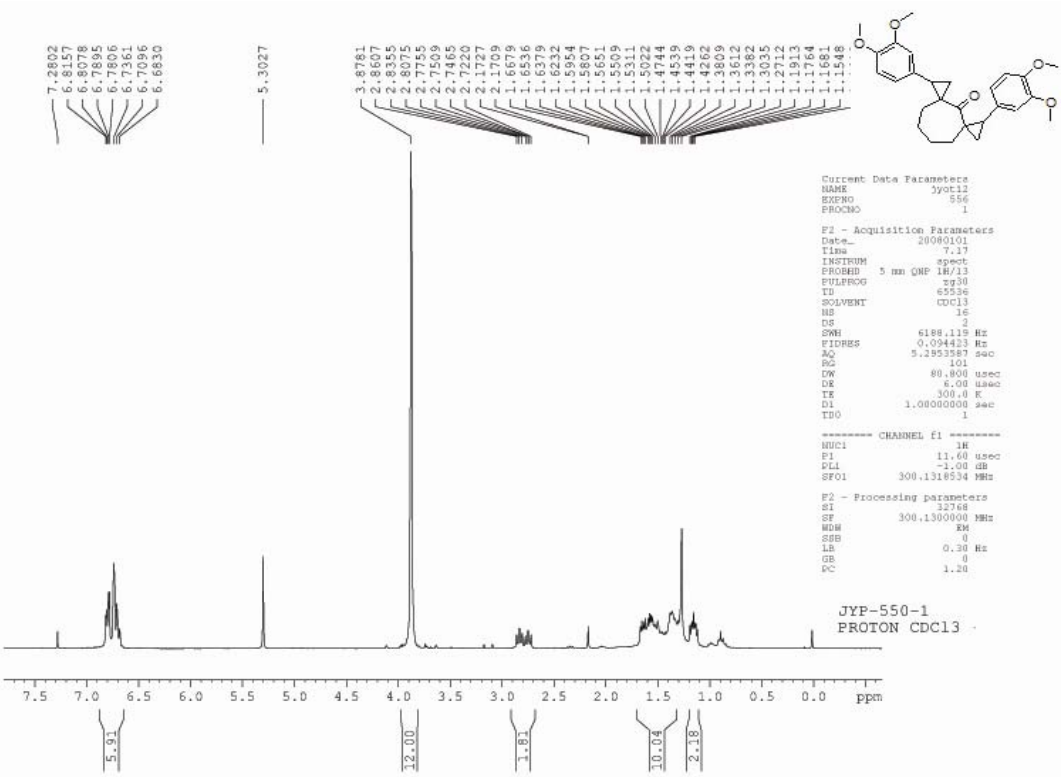
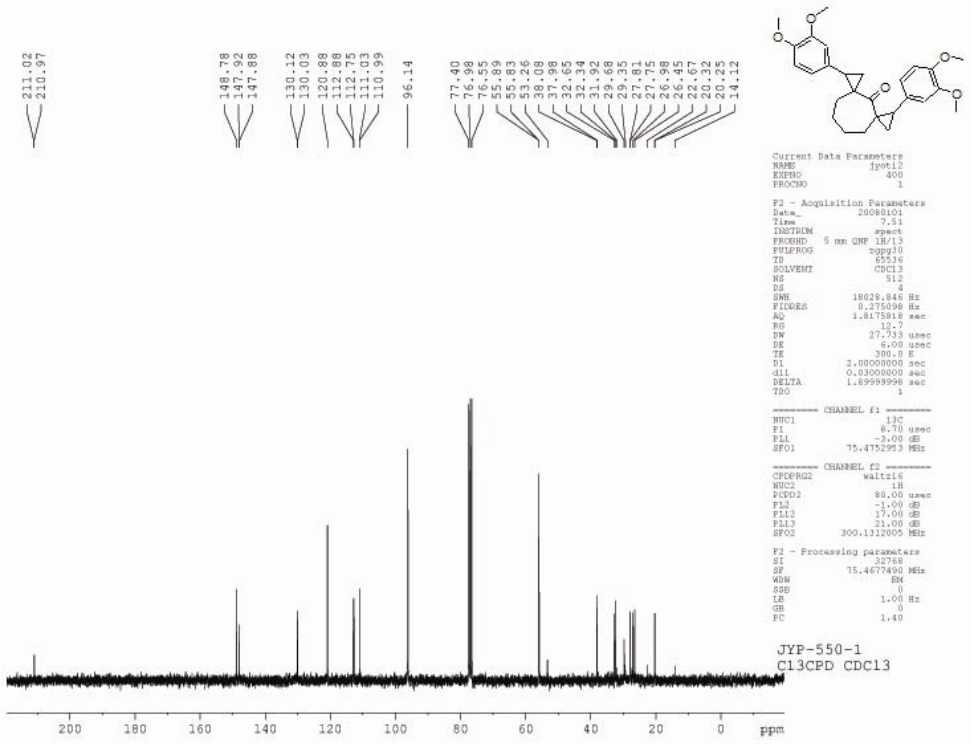


## $^{1}$H NMR of compound (**17**)




## $^{13}$C NMR of compound (**17**)



## Bioevaluation methods

### *In vitro* antituberculars assay (agar microdilution method)

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H37Rv was done by agar microdilution method where two fold dilutions of each test compound were added into 7H10 agar and *M. tuberculosis* H37Rv was used as test organism. The MIC of the test compounds was determined by incorporating lower concentrations of the test compound in middle brook 7H10 agar medium supplemented with OADC. A culture of *M. tuberculosis* H37Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. Suspensions of 1 $\mu gml^{-1}$ concentration of extracts/compounds soluble in dimethyl sulphoxide (DMSO) were prepared. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentration of the test compounds keeping the volume constant i.e. 0.1 mL. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv ($5 \times 10^4$ bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

### In silico screening

The three-dimensional structures of the compounds were built and optimized using the Builder module of Insight II (M/s Accelrys Inc.)[1]. All compounds were initially screened *in silico* by performing docking simulations using Autodock 4.0 as docking tool and the interaction energies

between the compounds and the proteins were calculated using the scoring function of same. The grid for docking calculations was centered on amino acid Lys123 which is an essential residue present in the binding site of LigA. The compounds with interaction energies greater than the control docking energies (calculated with natural ligand AMP) were selected for further enzymatic assays[2]. In order to identify compounds with specificity towards *Mtu*LigA, the complete cofactor binding site of *Mtu*LigA was used for all docking simulations. The complete $NAD^+$ binding site was modeled by superposing the individual subdomains (PDB: 1ZAU) in the structure onto the *E. faecalis* LigA-$NAD^+$ co-crystal structure (PDB: 1TAE). The virtual library was designed based on the dispiro cycloalkanones series of inhibitors and virtual screening was carried out using high throughput docking as the strategy. The compounds so filtered based on the docking energies were taken up further for the *in vitro* and *in vivo* assessment of the inhibitory activity (Table 3).

A retrospective linear regression analysis has also been performed to validate and check the robustness of the docking strategy and scoring function being used. A good correlation was found between the Autodock estimated $pK_i$ for all the known *Mtu*LigA inhibitors (Srivastava *et al.* 2005, 2007) and the $pIC_{50}$ via linear regression analysis and can be expressed by equation (eq. 1).

$$\textbf{Predicted Activity} = 2.61 + 0.381\ C2 \qquad \textbf{… eq. (1)}$$

where C2 is estimated Inhibition Constant ($K_i$), value of correlation coefficient ($r2$) is 0.544, value of standard deviation SD is 0.758

***In vitro* inhibition**

Nick sealing DNA ligation activities were carried out using a double-stranded 40 base pair DNA substrate carrying a single-strand nick as reported earlier [4]. The substrate was created in TE buffer by annealing 22-mer and 18-mer DNA complementary strands to a 40-mer (5′-ATG TCC AGT GAT CCA GCT AAG GTA CGA GTC TAT GTC CAG G-3′). At the 5′ end, the 18-mer was radio labeled with [$\gamma$-$^{32}$P]-ATP (3000 Ci/mmol, Board of Radiation and Isotope Technology, Mumbai) by using polynucleiotidyl kinase reaction. This labeled, nicked 40 bp DNA substrate was used to assay the *in vitro* inhibitory activity of different compounds against *Mtu*LigA, T4Lig, and HuLigI. Reaction was performed in buffer containing 50 mM Tris pH 8.0, 50 mM NaCl, 1mM DTT, and The $IC_{50}$ values were determined by plotting the relative ligation activity versus inhibitor concentration and fitting to the equation:

$$Vi/Vo = IC50/ (IC50+ [I]$$

using GraphPad Prism®. $V_0$ and $V_i$ represent rates of ligation in the absence and presence of inhibitor respectively and [I] refers to the inhibitor concentration.

***In vivo* assay**

The recombinant plasmid containing the gene for T4Lig in pTrc99A *(pRBL)* and *Mtu*LigA clone in *pTrc99A* both were transformed into *E. coli* GR501 ($lig_{251}$mutant Temperature sensitive strain). MIC values for the inhibitors were determined for *Mtu*LigA and T4Lig in *E. coli* GR501 $ligA^{ts}$ mutant along with *S. typhimurium* LT2 and its DNA ligase minus (null) mutant derivative, which had been rescued with a plasmid (pBR313/598/8/1b) encoding the T4Lig gene in order to check the specificity of compounds for $NAD^+$-dependent ligases from other sources as well. Antimicrobial activity was monitored in microtitre plates using micro dilution assay technique in a volume of 200 µL. Approximately $10^5$ CFU/mL in case of *E. coli* $LigA^{ts}$ mutant, $10^6$ CFU/mL

in case of *S. typhimurium* LT2 and its mutant LigA$^-$ strain, rescued with T4Lig, were incubated with different compound concentrations under ambient conditions for 20 h and MIC were determined on the basis of presence of any visible growth. *E. coli* mutant strain was grown in LB medium while nutrient broth was used for *S. typhimurium* strains. The media contained 20 μg/mL polymyxin B nonapeptide to facilitate passage of the inhibitors across the outer membrane.

**DNA-inhibitor interaction**

To probe the DNA intercalating properties of the inhibitors we used ethidium bromide displacement assay. Detection of ethidium bromide displacement from DNA, if any, is based on the strong loss in fluorescence that should occur upon its detachment from DNA[3]. The assay mixture contained in a volume of 100 μl, 5 μg of calf thymus DNA, 5 μM ethidium bromide, 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, and 1 mM EDTA and Inhibitor (0-250μM). Upon addition of the compounds in increasing concentrations, ethidium bromide fluorescence was immediately detected at an excitation wavelength of 485 nm and an emission wavelength of 612 nm.

## References

1. ACCELRYS ver., 11. San Diego, *CA, Accelrys Inc*., 2000, 92121-92152.
2. G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey and W. E. Hart, *J. Comput. Chem.*, 1998, **19**, 1639-1662.
3. J. B. Le-Pecq and C. Paoletti, *J. Mol. Biol.,* 1967, **27**, 87.
4. S. K. Srivastava, R. P. Tripathi and R. Ravishankar, *J. Biol. Chem.,* 2005, **280**, 30273-30281.