SUPPORTING INFORMATION

Cytoplasmic dynein antagonists with improved potency and isoform selectivity

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Figure S1. C7-benzyl ether ciliobrevins reversibly disrupt IFT88 trafficking. IFT88 distributions along the ciliary axoneme in response to treatment with analog 43 for 1 hour and then compound washout for 4 hours. Representative micrographs of an NIH-3T3 cell-derived line are shown with staining for IFT88, ARL13B (primary cilia), γ-tubulin (basal body), and DNA. Scale bar: 2 µm. Each axoneme was divided into 21 bins, and the IFT88 signal intensity within each bin was normalized to the total ciliary signal using Matlab R2014A (Mathworks). Data represent the average IFT88 signal intensities for 65 cilia ± s.e.m.
Figure S2. Effects of C7-benzyl ether ciliobrevins on Golgi morphology and GLI2 trafficking. Representative Golgi organization and GLI2 trafficking phenotypes observed in a serum-starved NIH-3T3 cell-derived line treated with DMSO or ciliobrevins for 4 hours. Cytoplasmic dynein 1 inhibition causes Golgi vesicle dispersion, whereas cytoplasmic dynein 2 blockade causes GLI2 accumulation at the distal tip of primary cilia (ARL13B). Scale bar: 5 µm. Quantitative analyses of the Golgi (top) and GLI2 (bottom) phenotypes are shown. GLI2 signal intensities at the ciliary tip were normalized to that of a SAG-treated control. At least 100 cells were scored for each condition, and GLI2 intensities are shown as average values ± s.e.m of three independent experiments.
Figure S3. ATP concentration-dependent inhibition of cytoplasmic dynein 1 and 2 motor domains by ciliobrevins. Dose-dependent inhibition of DYNC1H1 (black) and DYNC2H1 (red) motor ATPase activities by ciliobrevin analog 37 at 25 nM and 100 µM ATP concentrations. Data are the average of triplicate samples ± s.e.m.
Figure S4. Comparison of the nucleotide-binding sites in the cytoplasmic dynein 2 heavy chain. Stereoviews of the nucleotide-binding sites in DYNC2H1, visualized from their most solvent-accessible faces (PDB ID: ARH7). Space-filling (left) and surface (right) renderings are shown, and the linker region N-terminal to the AAA1 domain has been omitted for clarity. Adenine-interacting residues that are conserved between DYNC1H1 and DYNC2H1 are depicted in blue, variable residues in green, and nucleotides in red.
MATERIALS AND METHODS — SYNTHETIC PROCEDURES

Ciliobrevins A (1) and D (2) and analog 25 were synthesized as previously described. Ciliobrevins 3-19 and 23-31 were prepared using Procedure A, while analogs 20, 21, and 22 were obtained through separate routes. Benzyl ether derivatives 32-48 were prepared through Procedure B. Unless otherwise noted, all reactions were performed under a nitrogen atmosphere.

THF, DMF, and acetonitrile were distilled from calcium hydride and stored over 4 Å molecular sieves. Other reagents and solvents obtained from commercial sources were used directly without further purification. Purification of products was conducted by flash chromatography on silica gel (EM Science, 70-230 mesh; Sorbtech, 230-400 mesh; or Qingdao Haiyang Chemical, 200-300 mesh). 1H NMR spectra were recorded on Varian or Bruker NMR spectrometers, and chemical shifts are reported in δ units (ppm) using residual solvent as the internal standard. High-resolution mass spectra were obtained on quadrupole time-of-flight (Q-TOF) mass spectrometers utilizing the electrospray ionization method.

Representative procedure A for the synthesis of ciliobrevins 3-19 and 23-31

\[
\begin{align*}
\text{R}_1\text{H} & \xrightarrow{\text{2-cyanothioacetamide}} \text{NaOEt, EtBr, EtOH} \xrightarrow{\text{R}_1} \\
\text{O} & \quad \text{N} & \quad \text{CN}
\end{align*}
\]

2-(4-oxo-3,4-dihydroquinazolin-2-yl)acetonitrile derivatives. 2-cyanothioacetamide (200 mg, 2.00 mmol) and bromoethane (165 µL, 2.21 mmol) were added to an ethanolic solution of sodium ethoxide (2.00 mL, 2.20 mmol). The resulting mixture was stirred for 6 h. The corresponding substituted 2-aminobenzoic acid (2.00 mmol) was added, and the reaction was refluxed overnight with stirring. A solid precipitate formed upon cooling of the reaction mixture,
which was recovered by vacuum filtration and washed sequentially with ethanol, water, ethanol, and diethyl ether. The solid was then dried to yield the substituted 2-(4-oxo-3,4-dihydroquinazolin-2-yl) acetonitrile. Synthetic yields ranged from 30-50%.

**3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-phenylpropanenitrile derivatives.**

To a solution of 2-(4-oxo-1,4-dihydroquinazolin-2-yl)acetonitrile (92.5 mg, 0.499 mmol) and triethylamine (84 µL, 0.60 mmol) in dioxane (5 mL) was added the corresponding acyl chloride (0.500 mmol), and the resulting mixture was refluxed overnight with stirring. A solid precipitate formed upon cooling of the reaction mixture, which was recovered by vacuum filtration and washed sequentially with methanol, water, methanol, and dichloromethane. The solid was then dried to yield the desired product. Synthetic yields ranged from 30-70%.

**1H NMR and HRMS data for ciliobrevins 3-19 and 23-31**

**3-(2,4-dichlorophenyl)-2-(6-methyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (3).** 1H NMR (400 MHz, DMSO-<i>d</i><sub>6</sub>) δ 13.55 (br s, 1H), 7.85 (s, 1H), 7.73-7.75 (m, 2H), 7.65 (d, <i>J</i> = 8.4 Hz, 1H), 7.48-7.55 (m, 2H), 2.41 (s, 3H). HRMS (m/z) calc. for C<sub>18</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub> [M + H]<sup>+</sup>, 372.0307; observed, 372.0288.

**3-(2,4-dichlorophenyl)-2-(7-methyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (4).** 1H NMR (500 MHz, DMSO-<i>d</i><sub>6</sub>) δ 13.46 (br s, 1H), 7.94 (d, <i>J</i> = 8.0 Hz, 1H),
7.74 (s, 1H), 7.65 (s, 1H), 7.49-7.55 (m, 2H), 7.29 (d, \( J = 7.9 \) Hz, 1H), 2.44 (s, 3H). HRMS \((m/z)\) calc. for \(\text{C}_{18}\text{H}_{12}\text{Cl}_{2}\text{N}_{3}\text{O}_{2}\) \([\text{M + H}]^+\), 372.0307; observed, 372.0305.

3-(2,4-dichlorophenyl)-2-(6-ethyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (5). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.54 (br s, 1H), 7.87 (s, 1H), 7.70-7.79 (m, 3H), 7.50-7.56 (m, 2H), 2.73 (q, \( J = 7.2 \) Hz, 2H), 1.22 (t, \( J = 7.2 \) Hz, 3H). HRMS \((m/z)\) calc. for \(\text{C}_{19}\text{H}_{12}\text{Cl}_{2}\text{N}_{3}\text{O}_{2}\) \([\text{M – H}]^–\), 384.0307; observed, 384.0309.

3-(2,4-dichlorophenyl)-2-(7-ethyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (6). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.42 (br s, 1H), 7.95 (d, \( J = 8.0 \) Hz, 1H), 7.75 (d, \( J = 2.0 \) Hz, 1H), 7.71 (s, 1H), 7.49-7.56 (m, 2H), 7.33 (d, \( J = 8.4 \) Hz, 1H), 2.73 (q, \( J = 7.6 \) Hz, 2H), 1.22 (t, \( J = 7.6 \) Hz, 3H). HRMS \((m/z)\) calc. for \(\text{C}_{19}\text{H}_{12}\text{Cl}_{2}\text{N}_{3}\text{O}_{2}\) \([\text{M – H}]^–\), 384.0307; observed, 384.0312.

3-(2,4-dichlorophenyl)-2-(6-isopropyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (7). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.48 (br s, 1H), 7.96 (d, \( J = 8.1 \) Hz, 1H), 7.77-7.82 (m, 2H), 7.75 (d, \( J = 1.6 \) Hz, 1H), 7.49-7.56 (m, 2H), 2.49 (m, 1H), 1.24 (d, \( J = 6.8 \) Hz, 6H). HRMS \((m/z)\) calc. for \(\text{C}_{20}\text{H}_{14}\text{Cl}_{2}\text{N}_{3}\text{O}_{2}\) \([\text{M – H}]^–\), 398.0463; observed, 398.0467.

3-(2,4-dichlorophenyl)-2-(7-isopropyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (8). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 13.45 (br s, 1H), 7.96 (d, \( J = 8.1 \) Hz, 1H), 7.77 (d, \( J = 1.8 \) Hz, 2H), 7.50-7.58 (m, 2H), 7.41 (dd, \( J = 1.2, 8.1 \) Hz, 1H), 3.01-3.05 (m, 1H),
1.23 (d, \( J = 6.9 \) Hz, 6H). HRMS (m/z) calc. for C\(_{20}\)H\(_{14}\)Cl\(_2\)N\(_3\)O\(_2\) [M – H]\(^{–}\), 398.0463; observed, 398.0468.

2-(6-\textit{tert}-butyl-4-oxo-3,4-dihydroquinazolin-2(1\textit{H})-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (9). \(^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.22 (d, \( J = 2.0 \) Hz, 1H), 7.88 (dd, \( J = 2.4, 8.4 \) Hz, 1H), 7.50 (d, \( J = 1.6 \) Hz, 1H), 7.35-7.42 (m, 3H), 1.40 (s, 9H). HRMS (m/z) calc. for C\(_{21}\)H\(_{16}\)Cl\(_2\)N\(_3\)O\(_2\) [M – H]\(^{–}\), 412.0620; observed, 412.0626.

2-(7-\textit{tert}-butyl-4-oxo-3,4-dihydroquinazolin-2(1\textit{H})-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (10). \(^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.14 (d, \( J = 8.4 \) Hz, 1H), 7.50-7.55 (m, 2H), 7.34-7.42 (m, 3H), 1.40 (s, 9H). HRMS (m/z) calc. for C\(_{21}\)H\(_{16}\)Cl\(_2\)N\(_3\)O\(_2\) [M – H]\(^{–}\), 412.0620; observed, 412.0624.

3-(2,4-dichlorophenyl)-2-(6-methoxy-4-oxo-3,4-dihydroquinazolin-2(1\textit{H})-ylidene)-3-oxopropanenitrile (11). \(^1\text{H} \) NMR (400 MHz, DMSO-d\(_6\)) \(\delta\) 13.54 (br s, 1H), 7.80 (d, \( J = 9.6 \) Hz, 1H), 7.75 (s, 1H), 7.46-7.56 (m, 4H), 3.86 (s, 3H). HRMS (m/z) calculated for C\(_{18}\)H\(_{12}\)Cl\(_2\)N\(_3\)O\(_3\) [M + H]\(^{+}\): 388.0256; observed: 388.0233.

3-(2,4-dichlorophenyl)-2-(7-methoxy-4-oxo-3,4-dihydroquinazolin-2(1\textit{H})-ylidene)-3-oxopropanenitrile (12). \(^1\text{H} \) NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) 7.97 (d, \( J = 9.0 \) Hz, 1H), 7.77 (d, \( J = 2.0 \) Hz, 1H), 7.56 (dd, \( J = 8.1, 2.0 \) Hz, 1H), 7.52 (d, \( J = 8.1 \) Hz, 1H), 7.41 (br s, 1H), 7.05 (dd, \( J = 9.0, 2.2 \) Hz, 1H), 3.89 (s, 3H). HRMS (m/z) calculated for C\(_{18}\)H\(_{12}\)Cl\(_2\)N\(_3\)O\(_3\) [M + H]\(^{–}\): 388.0256; observed, 388.0241.
3-(2,4-dichlorophenyl)-2-(6-ethoxy-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (13). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.50 (s, 1H), 7.61 (d, $J = 2.0$ Hz, 1H), 7.34-7.44 (m, 4H), 7.26 (dd, $J = 2.8$, 8.8 Hz, 1H), 4.04 (q, $J = 7.2$ Hz, 2H), 1.35 (t, $J = 7.2$ Hz, 3H). HRMS ($m/z$) calc. for C$_{19}$H$_{12}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 400.0256; observed, 400.0261.

3-(2,4-dichlorophenyl)-2-(7-ethoxy-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (14). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 13.33 (br s, 1H), 7.93 (d, $J = 8.8$ Hz, 1H), 7.74 (d, $J = 2.0$ Hz, 1H), 7.49-7.56 (m, 2H), 7.37 (s, 1H), 7.00 (dd, $J = 2.4$, 8.8 Hz, 1H), 4.14 (q, $J = 7.2$ Hz, 2H), 1.38 (t, $J = 7.2$ Hz, 3H). HRMS ($m/z$) calc. for C$_{19}$H$_{12}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 400.0256; observed, 400.0258.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-6-propoxy-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (15). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 13.54 (br s, 1H), 7.76-7.82 (m, 2H), 7.45-7.57 (m, 4H), 4.04 (t, $J = 6.6$ Hz, 2H), 1.73-1.80 (m, 2H), 0.99 (t, $J = 7.2$ Hz, 2H). HRMS ($m/z$) calc. for C$_{20}$H$_{14}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 414.0412; observed, 414.0416.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-propoxy-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (16). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 13.37 (br s, 1H), 7.93 (d, $J = 9.0$ Hz, 1H), 7.77 (s, 1H), 7.50-7.57 (m, 2H), 7.40 (s, 1H), 7.01 (d, $J = 8.7$ Hz, 1H), 4.04 (d, $J = 6.6$ Hz, 2H), 1.75-1.82 (m, 2H), 0.99 (t, $J = 7.2$ Hz, 3H). HRMS ($m/z$) calc. for C$_{20}$H$_{14}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 414.0412; observed, 414.0420.
3-(2,4-dichlorophenyl)-2-(6-isopropoxy-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (17). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.43 (br s, 1H), 7.82 (d, $J = 9.6$ Hz, 1H), 7.75 (s, 1H), 7.69 (s, 1H), 7.49-7.56 (m, 2H), 7.44 (s, 2H), 4.74 (m, 1H), 1.29 (d, $J = 5.6$ Hz, 6H). HRMS (m/z) calc. for C$_{20}$H$_{14}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 414.0412; observed, 414.0416.

3-(2,4-dichlorophenyl)-2-(7-isopropoxy-4,3-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (18). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.33 (br s, 1H), 7.92 (d, $J = 9.2$ Hz, 1H), 7.76 (d, $J = 2.0$ Hz, 1H), 7.50-7.57 (m, 2H), 7.40 (d, $J = 1.6$ Hz, 1H), 6.98 (dd, $J = 2.4$, 8.0 Hz, 1H), 4.71 (m, 1H), 1.34 (d, $J = 6.0$ Hz, 6H). HRMS (m/z) calc. for C$_{20}$H$_{14}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 414.0412; observed, 414.0420.

(3-(2,4-dichlorophenyl)-2-(6-(dimethylamino)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (19). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.76-7.56 (m, 2H), 7.52 (q, $J = 1.6$, 8.2, 9.9 Hz, 2H), 7.35 (dd, $J = 2.8$, 9.3 Hz, 1H), 7.14 (d, $J = 2.7$ Hz, 1H), 3.00 (s, 3H). HRMS (m/z) calc. for C$_{19}$H$_{15}$Cl$_2$N$_4$O$_2$ [M + H]$^+$, 401.0567; observed, 401.0535.

3-(3,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (23). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.07 (d, $J = 7.8$ Hz, 1H), 7.92 (d, $J = 2.0$ Hz, 1H), 7.89 (t, $J = 7.8$ Hz, 1H), 7.86 (t, $J = 8.3$ Hz, 1H), 7.81 (d, $J = 8.3$ Hz, 1H), 7.70 (dd, $J = 2.0$, 8.3 Hz, 1H), 7.48 (t, $J = 7.8$ Hz, 1H). HRMS (m/z) calc. for C$_{17}$H$_{16}$Cl$_2$N$_3$O$_2$ [M + H]$^+$, 358.0145; observed, 358.0133.
3-(3-methoxyphenyl)-3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile
(24). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.03 (d, $J = 7.6$ Hz, 1H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.80 (t, $J = 8.0$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.39 (d, $J = 8.0$ Hz, 1H), 7.27 (d, $J = 7.6$ Hz, 1H), 7.22 (s, 1H), 7.12 (dd, $J = 1.6$, 8.0 Hz, 1H), 3.80 (s, 3H). HRMS ($m/z$) calc. for C$_{18}$H$_{14}$N$_3$O$_3$ [M + H]$^+$, 320.1035; observed, 320.1029.

3-oxo-2-(4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(pyridin-3-yl)propanenitrile
(26). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.02 (s, 1H), 8.85 (d, $J = 4.0$ Hz, 1H), 8.38 (d, $J = 8.0$ Hz, 1H), 8.05 (d, $J = 7.6$ Hz, 1H), 7.79-7.91 (m, 3H), 7.48 (t, $J = 7.6$ Hz, 1H). HRMS ($m/z$) calc. for C$_{16}$H$_{11}$N$_4$O$_2$ [M + H]$^+$, 291.0882; observed, 291.0865.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydrobenzo[g]quinazolin-2(1H)-ylidene)propanenitrile
(27). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.42 (br s, 1H), 8.79 (s, 1H), 8.20 (d, $J = 8.0$ Hz, 1H), 8.02 (d, $J = 8.4$ Hz, 1H), 7.76 (s, 1H), 7.71 (t, $J = 8.0$ Hz, 1H), 7.51-7.61 (m, 3H). HRMS ($m/z$) calc. for C$_{21}$H$_{12}$Cl$_2$N$_3$O$_2$ [M + H]$^+$, 408.0307; observed, 408.0318.

(3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,6,7,8,9-hexahydrobenzo[g]quinazolin-2(1H)-ylidene)propanenitrile
(28). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.93 (s, 1H), 7.49 (s, 1H), 7.30-7.40 (m, 2H), 7.12 (s, 1H), 2.85-2.96-4.40 (m, 4H), 1.85-1.94 (m, 4H). HRMS ($m/z$) calc. for C$_{21}$H$_{14}$Cl$_2$N$_3$O$_2$ [M – H]$^-$, 410.0463; observed, 410.0469.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,7,8-tetrahydro-[1,4]dioxino[2,3-g]quinazolin-2(1H)-ylidene)propanenitrile
(29). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.74 (d, $J = 2.0$ Hz, 1H),
7.53 (d, $J = 8.0$ Hz, 1H), 7.48 (d, $J = 8.0$ Hz, 1H), 7.43 (s, 1H), 7.34 (s, 1H), 4.33-4.40 (m, 4H).

HRMS ($m/z$) calc. for C$_{19}$H$_{10}$Cl$_2$N$_3$O$_4$ [M – H]$^-$, 414.0048; observed, 414.0054.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4,7,8-tetrahydro-1H-cyclopenta[g]quinazolin-2(6H)-ylidene)propanenitrile (30). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.44 (br s, 1H), 7.88 (s, 1H), 7.75 (d, $J = 1.6$ Hz, 1H), 7.69 (s, 1H), 7.49-7.56 (m, 2H), 2.96 (m, 4H), 2.06 (t, $J = 7.6$ Hz, 2H). HRMS ($m/z$) calc. for C$_{20}$H$_{12}$Cl$_2$N$_3$O$_2$ [M – H]$^-$, 396.0307; observed, 396.0305.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-3,4-dihydrofuro[3,4-g]quinazolin-2(1H,6H,8H)-ylidene)propanenitrile (31). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 13.55 (br s, 1H), 8.00 (s, 1H), 7.75-7.77 (m, 2H), 7.52-7.55 (m, 2H), 5.09 (s, 2H), 5.06 (s, 2H). HRMS ($m/z$) calc. for C$_{19}$H$_{10}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 398.0099; observed, 398.0102.

**Synthesis of ciliobrevin 20**

![Synthesis of ciliobrevin 20](image)

3-(2,4-dichlorophenyl)-2-(3-methyl-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (20). To a solution of ciliobrevin A (1) (107 mg, 0.299 mmol) in DMF (2 mL) was added sodium hydride (8.6 mg, 0.36 mmol) at 0°C, and the reaction mixture was stirred at room temperature for 40 min. To this solution was added iodomethane (47 mg, 0.33 mmol), and the resulting mixture was stirred at room temperature for 3 h. The reaction was quenched by addition of a saturated solution of NaHCO$_3$ (5 mL), and extracted with CH$_2$Cl$_2$ (3 × 20 mL). The
combined organic layers were washed with H$_2$O and brine, then dried over Na$_2$SO$_4$. The solvent was removed in vacuo and the residue was directly purified through preparative TLC to give the desired product (26 mg, 23%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 15.71 (s, 1H), 8.26 (d, $J$ = 8.0 Hz, 1H), 7.78 (dd, $J$ = 1.6, 8.0 Hz, 1H), 7.34-7.49 (m, 5H), 3.97 (s, 1H). HRMS ($m/z$) calc. for C$_{18}$H$_{12}$Cl$_2$N$_3$O$_2$ [M + H]$^+$, 372.0307; observed, 372.0290.

**Synthesis of ciliobrevin 21**

![Chemical structure](image)

**2-(2,4-dichlorobenzoyl)-3,3-bis(methylthio)acrylonitrile.** To a stirred solution of 3-(2,4-dichlorophenyl)-3-oxopropanenitrile (1.06 g, 4.95 mmol) in THF (15 mL) at 0 °C was added dry sodium hydride (0.24 g, 10 mmol). The suspension was stirred at 0 °C for 1 h, at which point carbon disulfide (0.403 g, 5.29 mmol) was added, and the reaction was then stirred at room temperature for another 2 h. The resulting red solution was cooled to 0 °C, iodomethane (1.56 g, 11.0 mmol) was added, and the mixture was stirred at room temperature for 18 h. The solvent was then removed in vacuo, and the residue was diluted in ether and washed with brine. The aqueous layer was extracted twice with ether, and the combined organic layers were washed twice with 5% sodium thiosulfate, and then brine. The organic layers were dried over MgSO$_4$, filtered and concentrated to give the desired acrylonitrile as a yellow powder (1.08 g, 68%). This product was used in the next step without further purification or characterization.
3-(2,4-dichlorophenyl)-2-(3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (21). A solution of 2-(2,4-dichlorobenzoyl)-3,3-bis(methylthio)acrylonitrile (0.954 g, 3.00 mmol) and 2-aminobenzylamine (366 mg, 3.00 mmol) in ethanol (10 mL) was heated to reflux for 4 h. After cooling the precipitate was filtered. Recrystallization from ethanol gave the desired product (380 mg, 37%). ^1H NMR (400 MHz, DMSO-^d_6) δ 11.06 (br s, 1H), 9.58 (br s, 1H), 7.69 (s, 1H), 7.50 (dd, J = 1.6, 8.4 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.09-7.24 (m, 4H), 4.59 (s, 2H). HRMS (m/z) calc. for C_{17}H_{12}Cl_{2}N_{3}O [M + H]^+, 344.0357; observed, 344.0344.

**Synthesis of ciliobrevin 22**

1-(2,4-dichlorophenyl)-2-methyl-3,3-bis(methylthio)prop-2-en-1-one. To a stirred solution of 1-(2,4-dichlorophenyl)propan-1-one (1.02 g, 5.02 mmol) in THF (15 ml) at 0 °C was added dry sodium hydride (0.24 g, 10 mmol). The suspension was stirred at 0 °C for 1 h, at which point carbon disulfide (0.403 g, 5.29 mmol) was added, and the reaction was then stirred at room temperature for another 2 h. The resulting red solution was cooled to 0 °C and iodomethane (1.56 g, 11.0 mmol) was added, and the mixture was stirred at room temperature for 18 h. The solvent was then removed *in vacuo*, and the residue was diluted in ether and washed with brine. The aqueous layer was extracted twice with ether, and the combined organic layers were washed
twice with 5% sodium thiosulfate, and then brine. The organic layers were dried over MgSO₄, filtered and concentrated to give the desired propenone, which was used in the next step without further purification or characterization.

![Chemical structure](image)

**2-(1-(2,4-dichlorophenyl)-1-oxopropan-2-ylidene)-2,3-dihydroquinazolin-4(1H)-one (22).** A solution of 1-(2,4-dichlorophenyl)-2-methyl-3,3-bis(methylthio) prop-2-en-1-one (307 mg, 1.00 mol) and 2-aminobenzamide (136 mg, 1.00 mmol) in xylene (10 mL) was heated to reflux for 3 h. After cooling, the precipitate was filtered. Recrystallization from ethanol gave the desired quinazolinone as colorless crystals (118 mg, 34%). ¹H NMR (400 MHz, CDCl₃) δ 15.65 (s, 1H), 8.44 (s, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.70 (dt, J = 1.6, 8.0 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.23-7.35 (m, 4H), 1.80 (s, 3H). HRMS (m/z) calc. for C₁₇H₁₃Cl₂N₂O₂ [M + H]⁺, 347.0354; observed, 347.0351.

**Representative procedure B for the synthesis of ciliobrevins 32-48**

![Chemical structure](image)

**3-(2,4-dichlorophenyl)-2-(7-hydroxy-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile.** To a solution of ciliobrevin 12 (104 mg, 0.268 mmol) in dry CH₂Cl₂ (15 mL) was
added 1.0 M BBr₃ in CH₂Cl₂ (2.6 mL, 2.6 mmol) at 0 °C. The reaction was allowed to warm to room temperature, continuously stirred for 7 days, and then quenched with water (100 mL). The mixture was then extracted with ethyl acetate (3 x 100 mL), and the organic layers were pooled, washed with brine, and concentrated *in vacuo*. The residue was then purified by silica gel chromatography (CHCl₃/MeOH/triethylamine, 8:1:1). Product containing fractions were combined and sequentially washed with 1N HCl, saturated NaHCO₃, and brine. Drying over MgSO₄ and removal of solvent *in vacuo* yielded the desired hydroxyquinazolinone (79.3 mg, 82%). ¹H NMR (500 MHz, CD₃OD) δ 6.88 (d, J = 8.8 Hz, 1H), 6.99 (s, 1H), 7.45 (m, 2H), 7.56 (d, J = 1.7 Hz, 1H), 8.00 (d, J = 8.3 Hz, 1H). HRMS (m/z) calc. for C₁₇H₁₀Cl₂N₃O₃ [M + H]⁺, 374.0099; observed, 374.0087.

**2-(7-(benzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile derivatives.** To a solution of 3-(2,4-dichlorophenyl)-2-(7-hydroxy-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (37 mg, 0.099 mmol) in DMF (4 mL) was added corresponding benzyl bromide (0.15 mmol) and K₂CO₃ (41 mg, 0.30 mmol), and the reaction was heated at 80 °C overnight with stirring. The mixture was then concentrated *in vacuo*, and the resulting residue was purified through flash chromatography (CH₂Cl₂/MeOH, 30:1) to afford the desired product. Synthetic yields ranged from 40-60%.

![Chemical structure of the target compound](image-url)
**H NMR and HRMS data for ciliobrevins 32-48**

2-(7-(2-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (32). H NMR (400 MHz, DMSO-d$_6$) δ 13.42 (br s, 1H), 8.01 (d, $J$ = 0.8 Hz, 1H), 7.75-7.43 (m, 8H), 7.16 (m, 1H), 5.30 (s, 2H). HRMS (m/z) calc. for C$_{24}$H$_{14}$Cl$_3$N$_3$O$_3$ [M – H]$^-$, 496.0022; observed, 496.0027.

2-(7-(3-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (33). H NMR (400 MHz, DMSO-d$_6$) δ 13.36 (br s, 1H), 8.00 (d, $J$ = 8.8 Hz, 1H), 7.76 (d, $J$ = 1.6 Hz, 1H), 7.56-7.45 (m, 7H), 7.15 (dd, $J$ = 2.0, 8.8 Hz, 1H), 5.26 (s, 2H). HRMS (m/z) calc. for C$_{24}$H$_{14}$Cl$_3$N$_3$O$_3$ [M – H]$^-$, 496.0022; observed, 496.0023.

2-(7-(4-chlorobenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (34). H NMR (400 MHz, DMSO-d$_6$) δ 13.33 (br s, 1H), 7.99 (d, $J$ = 9.2 Hz, 1H), 7.76 (d, $J$ = 1.6 Hz, 1H), 7.56-7.47 (m, 7H), 7.13 (dd, $J$ = 2.0, 8.8 Hz, 1H), 5.23 (s, 2H). HRMS (m/z) calc. for C$_{24}$H$_{14}$Cl$_3$N$_3$O$_3$ [M – H]$^-$, 496.0022; observed 496.0031.

3-(2,4-dichlorophenyl)-2-(7-(2-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (35). H NMR (400 MHz, DMSO-d$_6$) δ 13.40 (br s, 1H), 8.00 (d, $J$ = 8.8 Hz, 1H), 7.76 (d, $J$ = 1.6 Hz, 1H), 7.57-7.51 (m, 3H), 7.44 (d, $J$ = 7.2 Hz, 1H), 7.30-7.21 (m, 3H), 7.15 (dd, $J$ = 2.0, 8.8 Hz, 1H), 5.23 (s, 2H), 2.34 (s, 3H). HRMS (m/z) calc. for C$_{25}$H$_{17}$Cl$_2$N$_3$O$_3$ [M – H]$^-$, 476.0569; observed, 476.0574.
3-(2,4-dichlorophenyl)-2-(7-(3-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (36). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.34 (br s, 1H), 7.98 (d, \(J = 8.8\) Hz, 1H), 7.76 (d, \(J = 1.6\) Hz, 1H), 7.56-7.48 (m, 3H), 7.31-7.25 (m, 3H), 7.18 (d, \(J = 7.2\) Hz, 1H), 7.12 (dd, \(J = 2.4, 8.8\) Hz, 1H), 5.18 (s, 2H), 2.32 (s, 3H). HRMS (m/z) calc. for C\(_{25}\)H\(_{17}\)Cl\(_2\)N\(_3\)O\(_3\) [M – H]\(^-\), 476.0569; observed, 476.0572.

3-(2,4-dichlorophenyl)-2-(7-(4-methylbenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (37). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.36 (br s, 1H), 7.98 (d, \(J = 8.8\) Hz, 1H), 7.76 (d, \(J = 2.0\) Hz, 1H), 7.57-7.47 (m, 3H), 7.38 (d, \(J = 8.0\) Hz, 2H), 7.24 (d, \(J = 8.0\) Hz, 2H), 7.12 (dd, \(J = 2.4, 8.8\) Hz, 1H), 5.19 (s, 2H), 2.32 (s, 3H). HRMS (m/z) calc. for C\(_{25}\)H\(_{17}\)Cl\(_2\)N\(_3\)O\(_3\) [M – H]\(^-\), 476.0569; observed, 476.0571.

3-(2,4-dichlorophenyl)-2-(7-(2-methoxybenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (38). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 13.37 (br s, 1H), 7.96 (s, 1H), 7.77 (s, 1H), 7.55-7.38 (m, 5H), 7.13-7.00 (m, 3H), 5.19 (s, 2H), 3.84 (s, 3H). HRMS (m/z) calc. for C\(_{25}\)H\(_{16}\)Cl\(_2\)N\(_3\)O\(_4\) [M – H]\(^-\), 492.0518; observed, 492.0524.

3-(2,4-dichlorophenyl)-2-(7-(3-methoxybenzyloxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (39). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.54 (br s, 1H), 7.98 (d, \(J = 8.8\) Hz, 1H), 7.75 (s, 1H), 7.53-7.04 (m, 7H), 6.93 (m, 1H), 5.20 (s, 2H), 3.76 (s, 3H). HRMS (m/z) calc. for C\(_{25}\)H\(_{16}\)Cl\(_2\)N\(_3\)O\(_4\) [M – H]\(^-\), 492.0518; observed, 492.0520.
3-(2,4-dichlorophenyl)-2-(7-(4-methoxybenzyl)oxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (40). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.36 (br s, 1H), 7.97 (d, $J = 8.8$ Hz, 1H), 7.75 (s, 1H), 7.56-7.49 (m, 2H), 7.43 (d, $J = 8.4$ Hz, 3H), 7.08 (d, $J = 8.8$ Hz, 1H), 6.98 (d, $J = 8.8$ Hz, 1H), 5.15 (s, 2H), 3.76 (s, 3H). HRMS ($m/z$) calc. for C$_{25}$H$_{16}$Cl$_2$N$_3$O$_4$ [M – H]$^-$, 492.0518; observed, 492.0523.

2-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (41). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.39 (br s, 1H), 8.02 (d, $J = 8.8$ Hz, 1H), 7.96 (d, $J = 7.6$ Hz, 1H), 7.78-7.75 (m, 3H), 7.63-7.50 (m, 4H), 7.17 (dd, $J = 2.0$, 8.8 Hz, 1H), 5.40 (s, 2H). HRMS ($m/z$) calc. for C$_{25}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 487.0365; observed, 487.0369.

3-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (42). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.37 (br s, 1H), 8.00-7.97 (m, 2H), 7.87-7.83 (m, 2H), 7.77 (d, $J = 1.6$ Hz, 1H), 7.67 (t, $J = 7.6$, 15.2 Hz, 1H ), 7.57-7.48 (m, 3H), 7.16 (dd, $J = 2.0$, 8.8 Hz, 1H), 5.31 (s, 2H). HRMS ($m/z$) calc. for C$_{25}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 487.0365; observed, 487.0370.

4-(((2-(1-cyano-2-(2,4-dichlorophenyl)-2-oxoethylidene)-4-oxo-1,2,3,4-tetrahydroquinazolin-7-yl)oxy)methyl)benzonitrile (43). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 13.36 (br s, 1H), 8.00-7.90 (m, 3H), 7.76-7.66 (m, 3H), 7.54-7.47 (m, 3H), 7.15 (d, $J = 8.4$ Hz, 1H), 5.35 (s, 2H). HRMS ($m/z$) calc. for C$_{25}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 487.0365; observed, 487.0370.
3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-2-ylmethoxy)-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (44). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.41 (br s, 1H), 8.63 (dd, $J = 0.8$, 4.8 Hz, 1H), 8.00 (d, $J = 8.8$ Hz, 1H), 7.90 (m, 1H), 7.76 (d, $J = 2.0$ Hz, 1H), 7.56-7.38 (m, 5H), 7.16 (dd, $J = 2.4$, 8.8 Hz, 1H), 5.32 (s, 2H). HRMS (m/z) calc. for C$_{23}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 463.0365; observed, 463.0371.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-3-ylmethoxy)-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (45). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.57 (br s, 1H), 8.76 (s, 1H), 8.63 (d, $J = 4.4$ Hz, 1H), 8.01 (m, 2H), 7.75 (s, 1H), 7.55-7.45 (m, 4H), 7.12 (d, $J = 8.4$ Hz, 1H), 5.31 (s, 2H). HRMS (m/z) calc. for C$_{23}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 463.0365; observed, 463.0372.

3-(2,4-dichlorophenyl)-3-oxo-2-(4-oxo-7-(pyridin-4-ylmethoxy)-3,4-dihydroquinazolin-2(1H)-ylidene)propanenitrile (46). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.45 (br s, 1H), 8.66 (d, $J = 4.4$ Hz, 2H), 7.98 (d, $J = 8.8$ Hz, 1H), 7.71 (s, 1H), 7.56-7.46 (m, 4H), 7.24 (s, 1H), 7.08 (d, $J = 8.4$ Hz, 1H), 5.37 (s, 2H). HRMS (m/z) calc. for C$_{23}$H$_{14}$Cl$_2$N$_4$O$_3$ [M – H]$^-$, 463.0365; observed, 463.0372.

2-(7-(Benzo[d][1,3]dioxol-5-ylmethoxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-(2,4-dichlorophenyl)-3-oxopropanenitrile (47). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.33 (br s, 1H), 7.97 (s, 1H), 7.76 (s, 1H), 7.54-7.47 (m, 3H), 7.06-6.97 (m, 4H), 6.03 (s, 2H), 5.11 (s, 2H). HRMS (m/z) calc. for C$_{25}$H$_{15}$Cl$_2$N$_3$O$_5$ [M – H]$^-$, 506.0311; observed, 506.0318.
3-(2,4-dichlorophenyl)-2-(7-((4-(methylsulfonyl)benzyl)oxy)-4-oxo-3,4-dihydroquinazolin-2(1H)-ylidene)-3-oxopropanenitrile (48). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.35 (br s, 1H), 8.01-7.97 (m, 3H), 7.76-7.74 (m, 3H), 7.57-7.50 (m, 3H), 7.16 (dd, $J =$ 2.4, 8.8 Hz, 1H), 5.39 (s, 2H), 3.23 (s, 3H). HRMS ($m/z$) calc. for C_{25}H_{17}Cl_{2}N_{3}S $[M - H]^{-}$, 540.0188; observed, 540.0193.
MATERIALS AND METHODS — BIOCHEMICAL AND CELLULAR ASSAYS

Generation of stable DYNC1H1- and DYNC2H1-expressing cells

Flp-In T-Rex 293 cells (Invitrogen) were seeded at 2.5 million cells/15-cm plate in DMEM containing 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin and cultured for 22 h. A 500-μL solution of 0.25 M CaCl₂, 9.0 μg pOG44 (Invitrogen), and 1.0 μg of either SBP-SNAP-DYNConH1 or SBP-SNAP-DYNConH1 plasmids (which encode the full-length human cytoplasmic dynein 1 and 2 heavy chains with N-terminal streptavidin-binding peptide (SBP) and SNAP tags),³ was then slowly added to 500 μL HEPES buffered saline (280 mM NaCl, 1.5 mM Na₂HPO₄, 50 mM HEPES, pH 7.0) and allowed to sit for one minute. The resulting turbid solution was added dropwise to one plate of Flp-In T-Rex 293 cells, and the cells were cultured for 48 h with a media change at 24 h. The cells were then split into selection medium (DMEM containing 10% (v/v) FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 10 μg/mL blasticidin, and 100 μg/mL hygromycin B) and cultured to give colonies of stably-transfected cells.

Expression and purification of SBP-SNAP-DYNConH1 and SBP-SNAP-DYNConH1

Two hundred 15-cm plates of either SBP-SNAP-DYNConH1- or SBP-SNAP-DYNConH1-expressing Flp-In T-Rex 293 cells were cultured to 70% confluency in DMEM containing 10% (v/v) FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 10 μg/mL blasticidin, and 100 μg/mL hygromycin B. Doxycycline (2 μg/mL) was added, and the cells were cultured for an additional 48 h. The cells were then harvested via trypsinization and centrifugation at 200 g, resuspended in 200 mL of ice-cold lysis buffer (25 mM HEPES, 50 mM PIPES, 2 mM MgSO₄,
0.2 mM EGTA, 0.1% (v/v) Triton X-100, 1 mM DTT, 2 mM PMSF, Complete MiniProtease Inhibitor (Roche), pH 7.5), and sonicated on ice until the cells were completely homogenized. Lysates were cleared by centrifugation at 30,000 g for 30 min at 4 °C, and incubated with 5 mL of Streptavidin High Performance Beads (GE Healthcare) for 4 h at 4 °C. The matrix was washed with 100 mL of lysis buffer and added to a 1 cm x 15 cm glass Econo-Column (Bio-Rad), and the SBP-SNAP-DYNC1H1 or SBP-SNAP-DYNC2H1 protein was eluted in 25 x 500-µL aliquots with lysis buffer containing 2 mM biotin. The aliquots were analyzed on a 3-8% Tris-Glycine SDS-PAGE gel (Bio-Rad), and protein-containing fractions were pooled and dialyzed in 1 L of assay buffer (50 mM Tris-HCl, 150 mM KOAc, 2 mM Mg(OAc)₂, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, pH 8) overnight. Protein concentrations were quantified using Bradford reagent, and the purified heavy chains were snap-frozen in assay buffer containing 1% (v/v) glycerol using liquid nitrogen and stored at -80 °C.

**Expression and purification of 6xHis-DYNC1H1 and GFP-DYNC2H1 motor domains**

The motor domains of DYNC1H1 (amino acids 1320-4647) and DYNC2H1 (amino acids 1091-4307) were expressed in insect cells and purified as described below. In the case of the DYNC1H1 motor, an N-terminal 6x-His tag was used for affinity-based purification. Construct preparation was performed as follows: A human *DYNC1H1* cDNA clone (pF1KA0325) was obtained from Kazusa DNA Research Institute, and the coding region that encompasses the motor domain (residues Q1320 - V4647) was amplified and fused with a hexahistidine (His₆)-tag by PCR using Phusion High-Fidelity DNA Polymerase (NEB). The assembled construct was cloned into a pFastBac (Invitrogen) vector using an InFusion HD cloning Kit (Clontech Laboratories).
Purification of the DYNC1H1 motor domain proceeded as follows: An Sf9 cell pellet was resuspended in a buffer containing, 30mM HEPES pH 7.6, 200 mM NaCl, 10 mM imidazole, 1 mM TCEP, 2 mM PMSF, HALT (Thermo Fisher) and complete protease inhibitor cocktail (Roche) and lysed by the addition of Triton X-100 to a final concentration of 0.2% (v/v). The lysate was clarified by centrifugation at 140,000 g and then incubated with Ni-NTA beads for 2 h at 4 °C. The beads were washed with 75 bed volumes of lysis buffer, and bound proteins were eluted with buffer containing 30 mM HEPES pH 7.5, 100 mM NaCl, 500 mM imidazole, and 1 mM TCEP. Eluate fractions containing the DYNC1H1 motor domain were diluted into gel-filtration buffer (50 mM Tris HCl pH 7.8, 150 mM KOAc, 2 mM Mg(OAc)_2, 1 mM EGTA, 1 mM EDTA, 0.1 mM ATP, and 1 mM DTT) and loaded onto a Mono Q anion exchange column (GE Healthcare). The column was eluted using a salt gradient from 150 to 750 mM KOAc over 20 column volumes. Peak fractions were concentrated using a centrifugal concentrator (Amicon) and subjected to size-exclusion chromatography on a Superose 6 column using gel filtration buffer. Gel filtration revealed a monodisperse peak at an elution volume of 12.2 mL, consistent with the expected molecular weight. Peak fractions were pooled, supplemented with glycerol to a final concentration of 20% (v/v), and snap frozen. The protein yield was ~ 1 mg of DYNC1H1 motor domain per 1 L of Sf9 culture.

The DYNC2H1 motor domain was prepared as described previously. Briefly, the protein was expressed with N-terminal protein A tag and GFP tags separated by a tobacco etch virus (TEV) protease site and purified using IgG-Sepharose. The protein A tag was then proteolytically removed, and gel filtration of the product revealed a monodisperse peak at an elution volume of 12.6 mL, consistent with the expected molecular weight.
DYNC1H1 and DYNC2H1 ATPase assays

The ATPase activities of purified SBP-SNAP-DYNC1H1 and SBP-SNAP-DYNC2H1 proteins were measured through the kinetic hydrolysis of $\gamma^{32}$P ATP. Individual 25-µL reactions were prepared in 96-well plates, each containing assay buffer (50 mM Tris-HCl, 150 mM KOAc, 2 mM Mg(OAc)$_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, pH 8) supplemented with 0.056 µg/mL of SBP-SNAP-DYNC1H1 or SBP-SNAP-DYNC2H1, 0.1% (w/v) BSA, 0.1% (v/v) Triton X-100, and either a ciliobrevin analog or an equivalent amount of DMSO vehicle (4%, v/v). The reactions were initiated by the addition of 1.6 µCi $\gamma^{32}$P ATP (Perkin Elmer; final concentration of 18-26 nM) and incubated at 37 °C for 12 min on a thermocycler. A 2.5-µL aliquot of each reaction was subsequently mixed with 300 µL of charcoal suspension (0.6 M acetic acid, 2.5 mM KH$_2$PO$_4$, and 4% (w/v) activated charcoal) and centrifuged to obtain a supernatant containing the hydrolyzed $^{32}$P orthophosphate. A 100-µL fraction of this supernatant was added to 1 mL of Ultima Gold scintillation cocktail (Perkin Elmer) and $^{32}$P orthophosphate emissions were measured on a Beckman Coulter LS6500 liquid scintillation counter.

The ATPase activities of purified 6xHis-DYNC1H1 and GFP-DYNC2H1 motor domains were assayed in buffer containing 25 mM PIPES pH 7.0, 30 mM KCl, 1 mM EGTA, 5 mM MgCl$_2$, 0.01% (v/v) Triton X-100, 1 mM DTT, and 0.1 mg/mL BSA. For all conditions, the enzymes were incubated with varying concentrations of inhibitor in 2% DMSO for 10 minutes in a volume of 10 µL. Each ATPase reaction was initiated by addition of 2 µL of a 6x ATP stock containing trace $\gamma^{32}$P ATP (6000 Ci/mmol, 10 mCi/mL, Perkin Elmer) and allowed to proceed at room temperature for a time predetermined to lie within the linear range of the assay. For the “low ATP” conditions, final concentrations of 1 nM dynein motor and 25 nM ATP were used, and the reaction was allowed to proceed for 10 minutes. For the “high ATP” conditions, final
concentrations of 30 nM dynein and 100 μM ATP were used, and the reaction was allowed to proceed for 30 minutes. Reactions were quenched by the addition of 100 mM EDTA, and 2 μL of each reaction was spotted onto PEI-cellulose thin layer chromatography plates (Millipore). The plates were developed in a glass chamber with a freshly prepared solution of 150 mM formic acid and 150 mM LiCl, dried, exposed to a storage phosphor tray, and scanned on a Typhoon imaging system (GE Healthcare Life Sciences). The fraction of γ-phosphate hydrolyzed in each condition was quantified using ImageJ and normalized to a DMSO control.

**Hh signaling assays**

Shh-LIGHT2 cells, an NIH-3T3 cell line stably integrated with Gli-dependent firefly luciferase and thymidine kinase promoter-driven *Renilla* luciferase reporters, were cultured in DMEM containing 10% calf serum (CS), 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, 400 μg/mL G418, and 150 μg/mL zeocin. The cells were seeded at 35,000 cells/well in a 96-well plate and treated with individual ciliobrevin analogs or an equivalent amount of DMSO vehicle (1%, v/v) in DMEM containing 0.5% CS and 10% ShhN-conditioned medium. After 30 h, the cells were lysed, and their firefly and *Renilla* luciferase activities were measured using a Dual Luciferase Reporter kit (Promega) and a Veritas microplate luminometer. Dose-response data were curve-fitted with a variable slope, sigmoidal dose-response algorithm using Prism software (GraphPad) to obtain IC$_{50}$ values.

**Ciliogenesis assays**

Shh-EGFP cells, an NIH-3T3 cell line stably integrated with a Gli-dependent green fluorescent protein reporter, were cultured in DMEM containing 10% CS, 100 U/mL penicillin,
0.1 mg/mL streptomycin, 1 mM sodium pyruvate, and 150 µg/mL zeocin. The cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 35,000 cells/well and cultured for 40 h. The media was then replaced with DMEM containing 0.5% CS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mM sodium pyruvate, and either an individual ciliobrevin analog (30 µM) or an equivalent amount of DMSO vehicle (0.3%, v/v). Following a 24-h incubation, the cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 10 min at room temperature, washed 3 x 5 min with PBS, permeabilized with PBS containing 0.3% (v/v) Triton X-100 for 5 min, washed 2 x 5 min with PBS, and then blocked overnight at 4 °C in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were next treated overnight at 4 °C with blocking buffer containing mouse monoclonal anti-ARL13B antibody (1:1000; UC-Davis/NIH NeuroMab Facility, 75-287), washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, treated for 1 h at room temperature with blocking buffer containing AlexaFluor 488-conjugated goat polyclonal anti-mouse IgG antibody (1:400 dilution; Invitrogen, A-11029), washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, and mounted onto glass slides with Prolong Gold Antifade reagent containing DAPI (Invitrogen). The immunostained cells were then imaged on a Leica DMI6000B compound microscope equipped with an HC Pan Apochromat CS 20x/0.70 NA oil-immersion objective, a Photometric CoolSNAP HQ CCD camera, and Metamorph software (Molecular Devices).

To quantify primary cilia lengths, the minimum threshold intensity for cilia staining was first established by manual inspection, and ImageJ software was used to quantify the total pixel area of ARL13B-positive pixels equal to or greater than the minimum threshold intensity. The average cilia length was then determined by dividing the number of ARL13B-positive pixels by the number of DAPI-positive nuclei in each field of view, and at least 6 fields of view were
analyzed per experimental condition. Approximately 800 cells/condition were analyzed in this manner, although fewer cells were imaged in some cases due to decreased cell viability. Analogs 8, 12, 19, 23, and 40 could not be analyzed in this assay due to the formation of precipitates that interfered with fluorescence imaging.

**IFT88 trafficking assays**

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 125,000 cells/well and cultured for 24 h in the DMEM/10% CS medium described above. The cells were next cultured in DMEM containing 0.5% CS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin for 16 h to promote primary cilia formation, and then transferred into the low-serum medium containing either individual ciliobrevin analogs (50 µM) or an equivalent amount of DMSO vehicle (0.25%, v/v) for 1 h. After compound treatment, the Shh-EGFP cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 8 min at room temperature, washed 1 x with PBS before addition of ice-cold (-20 °C) methanol for 5 min at -20 °C. For wash-out experiments, cells were incubated with low-serum medium after compound treatment for 10 min, and transferred into fresh low-serum medium for an additional 4 h at 37 °C before fixing the cells as described above. Subsequently, cells were washed 1 x with PBS and 2 x with PBS containing 0.1% (v/v) Triton X-100, and blocked for 1 h at room temperature in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were subsequently treated with blocking buffer containing mouse monoclonal anti-ARL13B IgG2a antibody (1:3000; UC-Davis/NIH NeuroMab Facility, 75-287) overnight at 4 °C, washed 1 x 5 min in PBS containing 0.1% (v/v) Triton X-100, and then incubated with blocking buffer containing rabbit polyclonal anti-IFT88 antibody (1:70 dilution; ProteinTech
Group, 13967-1-AP) and mouse monoclonal anti-γ-Tubulin IgG1 antibody (1:500 dilution; Sigma, T6557, clone GTU-88, ascites fluid) for 90 min at room temperature. The immunostained cells were washed 4 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated in blocking buffer containing Alexa Fluor 488-conjugated goat polyclonal anti-rabbit IgG antibody (1:300 dilution; Invitrogen, A-11034), Alexa Fluor 594-conjugated goat polyclonal anti-mouse IgG1 antibody (1:500 dilution; Jackson ImmunoResearch, 115-585-205), Alexa Fluor 647-conjugated goat polyclonal anti-mouse IgG2a antibody (1:500 dilution; Jackson ImmunoResearch, 115-605-206) for 1 h at room temperature. Following the secondary antibody incubation, cells were washed 5 x 5 min with PBS containing 0.1% (v/v) Triton X-100. The coverslips were mounted onto glass slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen) to stain nuclei and imaged at 500-nm z intervals on a Zeiss microscope (Axio Imager.M1) using epifluorescent illumination (Lambda XL light source; Sutter Instrument) through a 63x/1.4 NA Plan Apochromat objective. Images were captured with a camera (CoolSNAP HQ²; Photometrics) using SlideBook software (Intelligent Imaging Innovations).

Quantitative image analyses were conducted using MatLab R2014A (Mathworks). ARL13B staining was used to mask and track the cilia and the γ-tubulin staining was used to orient the cilia from base to tip. The IFT88 signal along each axoneme was analyzed by dividing the total length of the cilium (as measured from the ARL13B staining) in 21 bins, each consisting of a 2-pixel-radius circle. Overlap correction was used to make sure that the summed fluorescence intensities over the 21 bins did not exceed the total ciliary fluorescence signal. The IFT88 signal within each bin was then normalized to the total ciliary signal to determine the fraction of ciliary IFT88 protein localized to each position along the axoneme. IFT88 signals
from 70-120 cilia were analyzed from 5 fields of view to obtain traces for each compound, and the experiments were performed in duplicate.

To assess the effects of ciliobrevins on IFT88 movement in real time, murine inner medullary collecting duct (IMCD3) cells stably expressing mNeonGreen-IFT88 were imaged as previously described. The cells were seeded on 25-mm coverslips and serum-starved for 24 h to induce ciliation. Imaging was conducted in phenol red-free media and on a DeltaVision system (Applied Precision) equipped with a PlanApo 603/1.49 NA internal reflection microscopy (TIRF) oil-immersion objective (Olympus). Images were captured with an sCMOS camera (Applied Precision) at 2 Hz, and line scan kymographs were generated by ImageJ. Velocities and frequencies of mNeonGreen-IFT88 foci movements were then quantified from the kymographs.

**Mitotic spindle morphology assays**

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 60,000 cells/well and cultured for overnight in the DMEM/10% CS medium described above. The cells were then cultured in the same growth medium containing 15 µM MG132 for 90 min, following by a 30-min incubation with DMEM containing 0.5% CS, 15 µM MG132, and either individual ciliobrevin analogs or an equivalent amount of DMSO vehicle (0.3%, v/v). The Shh-EGFP cells were treated with methanol chilled to -20 °C for 10 min, washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100, and blocked for 1 h at room temperature with PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The cells were subsequently incubated with blocking buffer containing mouse monoclonal anti-α-tubulin antibody (1:2000 dilution, Sigma-Aldrich, T6199) and rabbit polyclonal anti-γ-tubulin antibody (1:1500 dilution, Sigma-Aldrich, T3559) overnight at 4 °C. The immunostained cells were
washed 3 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated with blocking buffer containing Alexa Fluor 488-conjugated goat polyclonal anti-rabbit IgG antibody (1:400 dilution; Invitrogen, A-11034) and Alexa Fluor 594-conjugated goat polyclonal anti-mouse IgG antibody (1:400 dilution; Invitrogen, A-11032) for 1 h at room temperature. The coverslips were washed 3 x 5 min in PBS containing 0.1% (v/v) Triton X-100, mounted onto slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen), and imaged using a Zeiss LSM700 confocal laser-scanning microscope equipped with a Plan Apochromat 63x/1.4 NA oil-immersion objective. At least 200 mitotic spindles were analyzed for each experimental condition.

**Golgi dispersion and GLI2 trafficking assays**

Shh-EGFP cells were seeded onto poly-D-lysine-coated, 12-mm glass coverslips in 24-well plates at a density of 125,000 cells/well and cultured for 24 h in the DMEM/10% CS medium described above. The cells were next cultured in DMEM containing 0.5% CS, 100 U/mL penicillin, and 0.1 mg/mL streptomycin for 16 h to promote primary cilia formation, and then transferred into the low-serum medium containing either individual ciliobrevin analogs (5, 10, 20, or 30 µM) or an equivalent amount of DMSO vehicle (0.25%, v/v) for 4 h. After compound treatment, the Shh-EGFP cells were fixed in PBS containing 4% (w/v) paraformaldehyde for 10 min at room temperature, washed 2 x 5 min with PBS, permeabilized for 5 min with PBS containing 0.3% (v/v) Triton X-100 and washed 3 x 5 min with PBS. Subsequently, coverslips were blocked for 1 h at room temperature in PBS containing 1% (w/v) BSA and 0.1% (v/v) Triton X-100. The coverslips were subsequently treated with blocking buffer containing mouse monoclonal anti-ARL13B IgG2a antibody (1:3000 dilution; UC-
Davis/NIH NeuroMab Facility, 75-287) and goat polyclonal anti-GLI2 antibody (1:100 dilution; R&D systems, AF3635) overnight at 4 °C, washed 1 x 5 min in PBS containing 0.1% (v/v) Triton X-100, and then incubated with blocking buffer containing rabbit monoclonal anti-GM130 antibody (1:500 dilution; Abcam, ab52647) for 90 min at room temperature. The immunostained cells were washed 4 x 5 min with PBS containing 0.1% (v/v) Triton X-100 and incubated in blocking buffer containing Alexa Fluor 488-conjugated donkey polyclonal anti-goat IgG antibody (1:300 dilution; Invitrogen, A-11055), Alexa Fluor 594-conjugated donkey polyclonal anti-mouse IgG antibody (1:300 dilution; Invitrogen, A21203), Alexa Fluor 647-conjugated donkey polyclonal anti-rabbit IgG antibody (1:300 dilution; Invitrogen, A-31573) for 1 h at room temperature. Following the secondary antibody incubation, cells were washed 5 x 5 min with PBS containing 0.1% (v/v) Triton X-100. The coverslips were mounted onto glass slides using Prolong Gold Antifade reagent containing DAPI (Invitrogen) to stain nuclei and imaged at 500-nm z intervals on a Zeiss microscope (Axio Imager M1) using epifluorescent illumination (Lambda XL light source; Sutter Instrument) and a 63x/1.4 NA Plan Apochromat objective. Images were captured with a CoolSNAP HQ² camera (Photometrics) using SlideBook software (Intelligent Imaging Innovations).

Quantitative image analyses were conducted using MatLab R2014A (Mathworks). Golgi dispersal was analyzed by scoring specific morphologies as described in Figure S2. ARL13B staining was used to mask and track individual cilia, and GLI2 staining was used to designate the distal tip of each cilium. Each axenome was divided in 21 bins as described above, and the absolute fluorescent GLI2 signal at the ciliary tip (bin 19; maximum GLI2 signal) was averaged over 100-300 cilia analyzed from 4-6 fields of view. The GLI2 signal for each compound was
then normalized to that observed in cells treated with the Hh pathway activator SAG. Three independent experiments were analyzed.
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Compound 06

NOESY 400M

Current Experiment Parameters
NAME          HPI-95
EXPNO         1
PROCNO        1

F2 - Acquisition Parameters
Date_          20130427
Time_          12.05
INSTRTM       spect
PBD         5 mm PARBO BB-
PULPROG        zg30
TD            65536
SOLVENT       DMSO
NS            8
DS            2
SWH          8012.820 Hz
FDRES        0.122266 Hz
AQ           4.0894565 sec
RS           135.79
DW            62.400 use
DE            6.50 use
TE            0 K
DL         1.00000000 sec

====== CHANNEL f1 ======
NUC1        1H
Pl           15.00 use
PLW1      12.00000000 W
SP01       400.1326003 MHz

Water

DMSO
Compound 08

Water

DMSO

20130424-HPI-96

NAME 20130424-HPI-95
EXFNO 4
PROCNO 21
Date 20130424
Time 10.50
INSTRUM spect
PROBHD 5 nm FADUL 13C
PULPROG zg
TB 55576
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FTIDRES 0.091480 Hz
AQ 5.4637526 sec
RG 101
DW 83.400 usec
DE 6.50 usec
TE 292.2 K
DI 2.00000000 usec
TD0 1

-------- CHANNEL f1 --------
NUCL 1H
Fl 11.25 usec
PLL 0.00 dB
PL1W 8.31434441 W
SF11 300.1316458 MHz
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Compound 10

N
NH
CN
O
Cl
Cl
O
t-Bu
TMS
CDCl₃

20120517-HPI-082

1.405
1.384

14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

0.84 1.03 2.11 3.47 9.00
Compound 13

DMSO

Water

TMS

ppm

1.00

4.29

1.05

2.19

3.45

7.518

7.813

7.449

7.445

7.429

7.424

7.406

7.390

7.363

7.362

7.340

7.268

7.251

7.246

7.238

4.113

4.095

4.078

4.061

4.036

4.017

3.330

2.509

2.504

2.500

2.495

2.491

1.986

1.376

1.359

1.325

1.341

20130530-HPI-097

50
Compound 14
Compound 17

Current Data Parameters
- NAME: 20130607-HPI-101
- EXPERIMENT: 3

F2 - Acquisition Parameters
- Date: 20130607
- Time: 11:04
- INSTRUM: spect
- PROBED: 5 mm PARSH R-B
- PROBPROG: mp10
- TD: 65526
- SOLVENT: DMSO
- NR: 8
- DR: 2
- SW: 6012.820 Hz
- FIDRES: 0.122266 Hz
- AQ: 4.484986 sec
- EZ: 295.79
- DW: 6.56 sec
- TE: 299.2 K
- EL: 1.60000000 sec

------- CHANNEL 1 -------
- MFC1: 1B
- FL: 15.40 sec
- FWM1: 12.00000000 Hz
- FPU1: 400.1326009 Hz

F2 - Processing parameters
- SI: 15816
- SP: 400.1326009 Hz
- MOW: 1M
- SUB: 0
- LR: 0.10 Hz
- GC: 1.00

DMSO
Water
TMS
Compound 18

DMSO

TMS

Water
Compound 19
Compound 20

NOESY 400M
20111116-2

Current Data Parameters
NAME 2011116-2
EXENO 1
PRECOM 1

T2 - Acquisition Parameters
Date_ 20111116
Time 15:35
INSTAN spect
PSCNCOH 5 mm PABBO BB-
PULPROG 330
TO 651.16
SOLVENT CDCl3
WS 9
DS 2
SW 800.220 K
TURFS 0.12524 K
AQ 4.000000000000000000
R2 120.19
DW 62.410
DE 6.00
TE 296.8
E1 1.000000000000000000

-------- CHANNEL [1] -----
WFL 1
P M 15.70
PLM 12.000000000000000000
SCL 406.133600000000000000

T2 - Processing parameters:
S1 65356
SF 400.133600000000000000
WAV EPS
SNM 0
LB 0.30
GS 0
PC 1.00

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

Water
CDCl3
TMS
**Compound 21**

![Compound 21 structure](image)

**20111017-HPI-016**

**Current Data Parameters**
- **NAME**: 20111017-HPI-016
- **EXPERIMENT**: 1
- **PROCEDURE**: 1

**FT - Acquisition Parameters**
- **Date**: 20111017
- **Time**: 9.53
- **INSTRUM**: spect
- **PROBID**: 5 mm PAIRRO 88-
- **PULIGN**: 20.00
- **TD**: 65336
- **GOL/PC**: DMSO
- **M**: 8
- **Q**: 2
- **SN**: 8233.00 Hz
- **FIDRES**: 0.125483 Hz
- **AQ**: 3.9846177 μsec
- **NG**: 111.21
- **DN**: 1.000000 sec
- **DS**: 0.50 μsec
- **TS**: 294.8 μsec
- **DI**: 1.000000 μsec

**-------- CHANNEL f1 --------**
- **NUC**: 14
- **FI**: 15.00 μsec
- **FLW**: 12.000000 μsec
- **FID**: 406.132719 MHz

**F2 - Processing parameters**
- **DI**: 65336
- **DF**: 406.130000 MHz
- **DW**: 10.00 EM
- **DS**: 0.30 Hz
- **GS**: 0
- **TC**: 1.00

**--- ppm ---**

- **8.78**
- **8.85**
- **10.00**
- **2.056**
- **2.102**
- **1.995**
- **2.085**

**-----**

**--- ppm ---**

- **9.88**
- **3.279**
- **2.491**

**---**

**--- ppm ---**

- **12**
- **11**
- **10**
- **9**
- **8**
- **7**
- **6**
- **5**
- **4**
- **3**
- **2**
- **1**

**---**

**--- ppm ---**

- **58**
Compound 22

\[
\begin{align*}
\text{N} & \quad \text{NH} \\
\text{O} & \quad \text{Cl} \\
\text{O} & \quad \text{Cl}
\end{align*}
\]

Water

TMS

CDCl₃

1.00

1.02 1.01 1.03 1.09

17.05

59
Compound 24

HPI-027

Current Data Parameters
NAME 20110907-1
EXPIRE 1
PROCNO 1

FT - Acquisition Parameters
Puls 20.30957
Time 15.14
UNIFORM spectrum
P200D: 5 mm PFA 88-
PULPROG ez90
TD 453.36
SOLVENT DMSO
GS 0
SS 2
SNH 8012.8220
RTDS 0.123266
AQ 4.085446
BG 27.33
DN 62.100
DS 6.50
DS 298.31
D1 1.00000000

--- CHANNEL 71 ---
ZU1 1H
SI 15.00
P41 12.00000000
ZP1 406.132629001

Water
DMSO
Compound 31

NAME 20130413-HPI-92
EXPNO 21
PROCNO 1
Date 20130412
Time 14:45
INSTRUM spect
PROBHD 5 mm PADUL 13C
PULPROG zg
TD 65535
SOLVENT DMSO
NS 8
DS 0
SWH 5995.204 Hz
FIDRES 0.091480 Hz
AQ 5.4657525 sec
RG 255
DW 83.400 usec
DE 6.50 usec
TE 291.5 K
D1 2.00000000 sec
TDO 1

======== CHANNEL f1 ========
NUCl 1H
F1 11.25 usec
PL1 0.00 dB
PL1W 8.31434411 W
SPCI 300.1318458 MHz
SI 32768
SF 300.13000000 MHz
W1W EM
SSB 0
LB 0.30 Hz
GB 0
FC 1.00

Water
DMSO

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
1.0 1.8

68
Compound 32
Compound 34

Current Data Parameters
NAME: 20131109-HPI-106

Acquisition Parameters
Data: 20131109-HPI-106
Time: 16:01
INSTRIM: Agilent
PRIMED: 5 mm PABOR SE
SOLVENT: DMSO

DMSO
Water
TMS

--- CHANNEL 1 ---
reso 15.00 ppm

--- CHANNEL 2 ---
reso 13.40 ppm

--- CHANNEL 3 ---
reso 13.00 ppm

--- CHANNEL 4 ---
reso 13.60 ppm

--- CHANNEL 5 ---
reso 13.80 ppm

--- CHANNEL 6 ---
reso 14.00 ppm

--- CHANNEL 7 ---
reso 14.20 ppm

--- CHANNEL 8 ---
reso 14.40 ppm

--- CHANNEL 9 ---
reso 14.60 ppm

--- CHANNEL 10 ---
reso 14.80 ppm
Compound 36

Water

DMSO

TMS

73
Compound 39

DMSO

Water

Current Data Parameters

Date: 20131111

Processing parameters

0.0009056 MHz

0.30 Hz

1.00
Compound 40

N
NH
CN
O
Cl
O
O

Water
DMSO
TMS

1.29
1.00
2.24
2.92
1.24
2.13
2.10
3.31

5.146
3.762
3.356
2.504
2.500
2.498

2013III-HPi-109
Compound 45

Current Data Parameters
NAME  20131202-HPI-114
EXPNO  1
PROCNO  1

F2 - Acquisition Parameters
Date_ 20131202
Time  15.32
INSTRUM  spect
PROBHD  5 mm PABBO BB-
PULPROG  zg30
TD  65536
SOLVENT  DMSO
NS  8
DS  2
SNH  8013.820 Hz
FIDKES  0.12266 Hz
AQ  4.0894966 sec
AB  195.79
DW  62.400 usec
DE  6.50 usec
T2  296.5 K
DI  1.00000000 sec

****** CHANNEL f1 ******
NUCL  1H
PI  15.00 usec
PLW1  12.00000000 W
SPG1  400.132608 MHz

F2 - Processing parameters
SE  65536
SF  400.1300017 MHz
WDW  EM
SBB  0
LB  0.30 Hz
GB  1.00
Compound 46

DMSO

Water

83
