**Supporting information about**

# Synthesis and antiproliferative activity of 7-azaindirubin-3´-oxime, a 7-aza isostere of the natural indirubin pharmacophore

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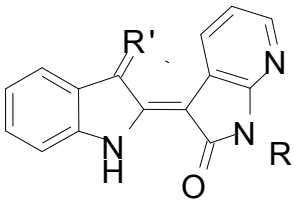
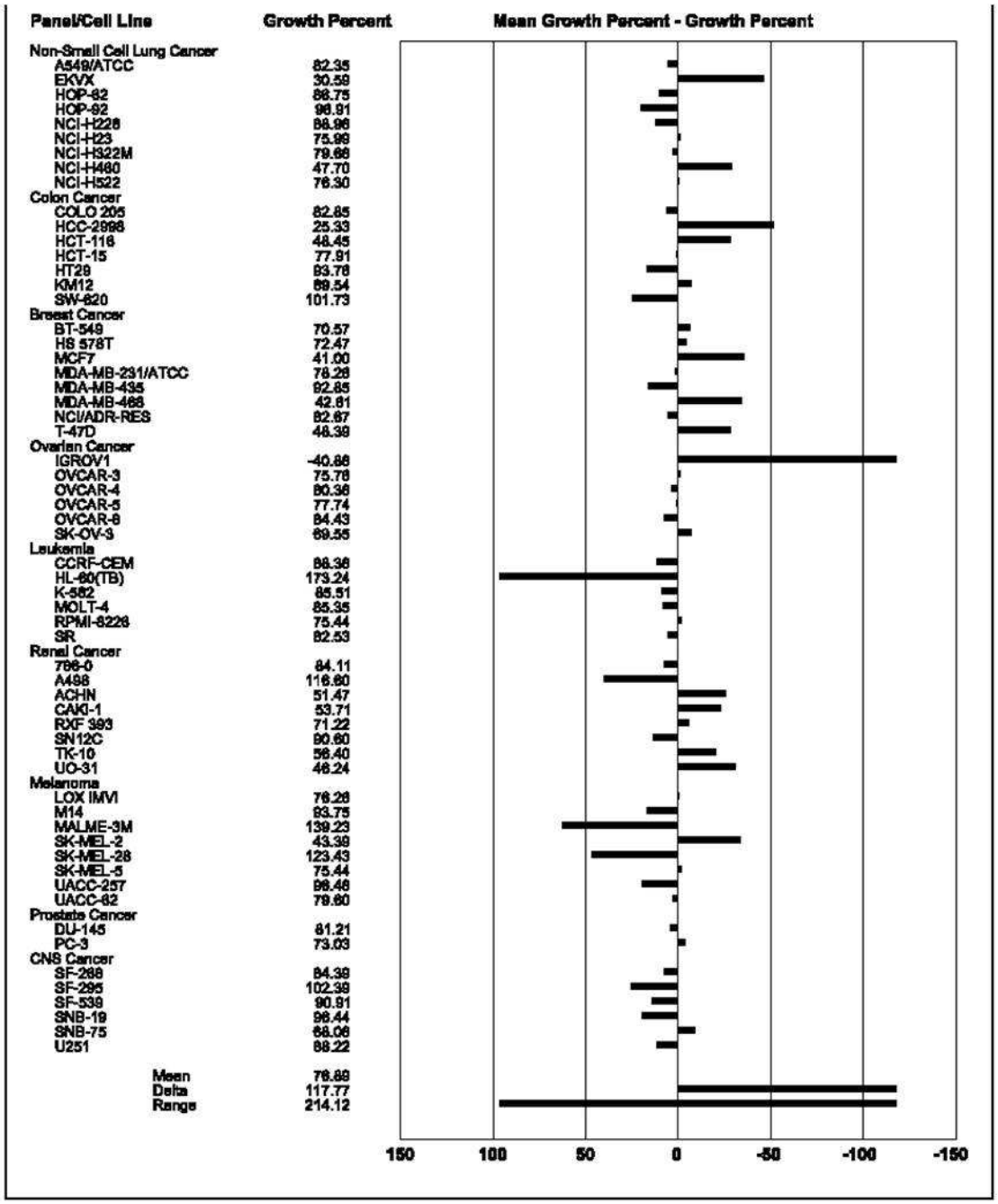
**Supplementary Table 1:** Evaluation of synthesized aza-indirubins as kinase inhibitors *in vitro* .



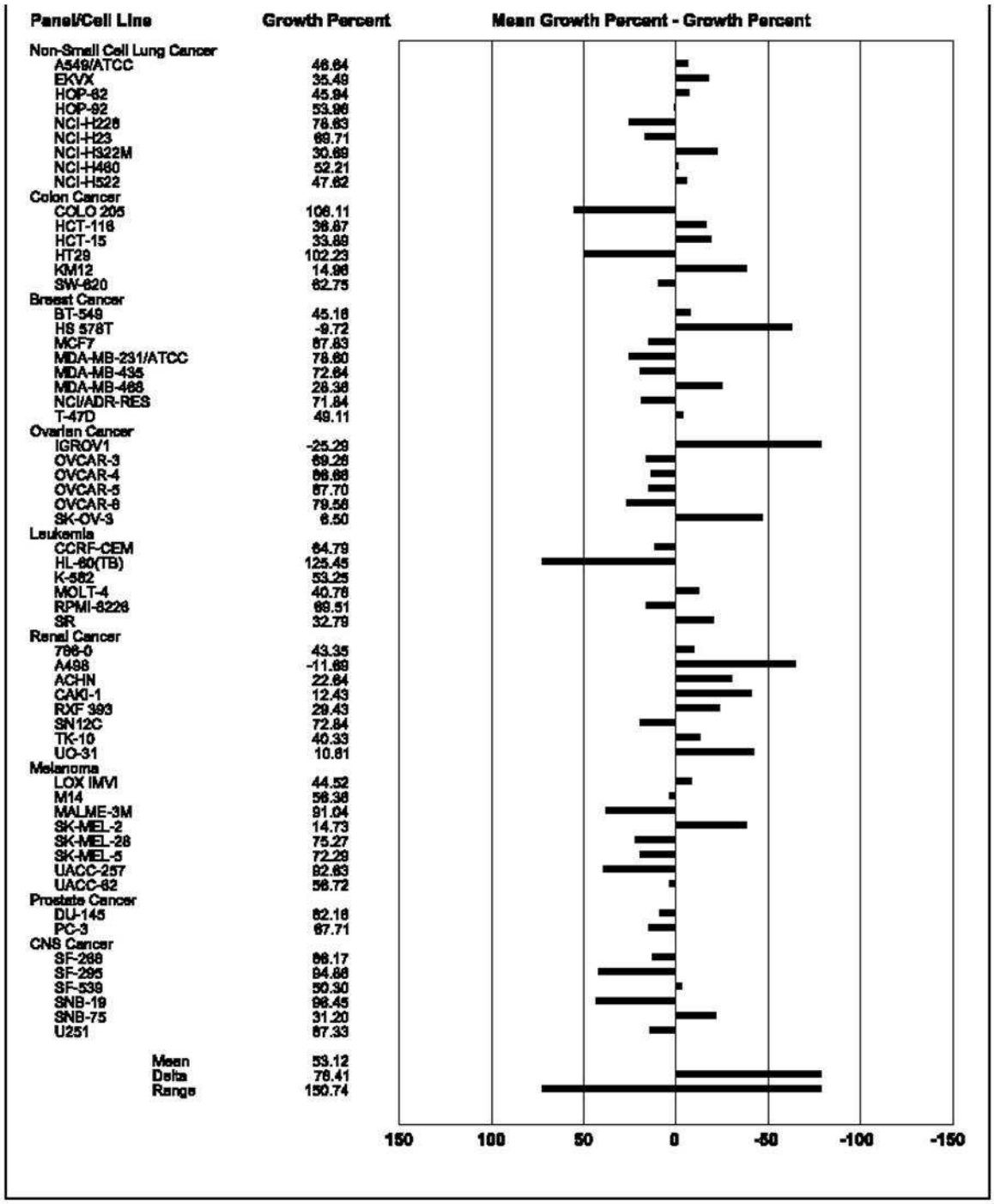

| Compound | R | R' | CDK1/ cyclin B | CDK2/ cyclin A | CDK5/ p35 | GSK–3*β* | ConceNAration tested (µM) |
|---|---|---|---|---|---|---|---|
| **10** | H | O | NA | NA | NA | 96 | 0.01 |
| | | | 88 | 99 | 82 | 93 | 0.3 |
| | | | 65 | 64 | 39 | NA | 3 |
| **12** | H | NOH | NA | NA | NA | 88 | 0.01 |
| | | | 105 | 100 | 65 | 64 | 0.3 |
| | | | 69 | 76 | 19 | 18 | 3 |
| **11** | $CH_3$ | O | NA | NA | NA | 101 | 0.01 |
| | | | 107 | 112 | 95 | 96 | 0.3 |
| | | | 105 | 106 | 107 | NA | 3 |
| **13** | $CH_3$ | NOH | NA | NA | NA | 95 | 0.01 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 109 | 109 | 108 | 89 | 0.3 |
| | | | 97 | 116 | 90 | NT | 3 |
| **7** | - | - | NA | NA | NA | 94 | 0.01 |
| | | | 88 | 114 | 101 | 105 | 0.3 |
| | | | 95 | 109 | 84 | NA | 3 |
| **6BIO** | - | - | NA | NA | NA | 66 | 0.01 |
| | | | 85 | 55 | 29 | 7 | 0.3 |
| | | | 21 | 8 | 6 | NA | 3 |

NA: Not assayed

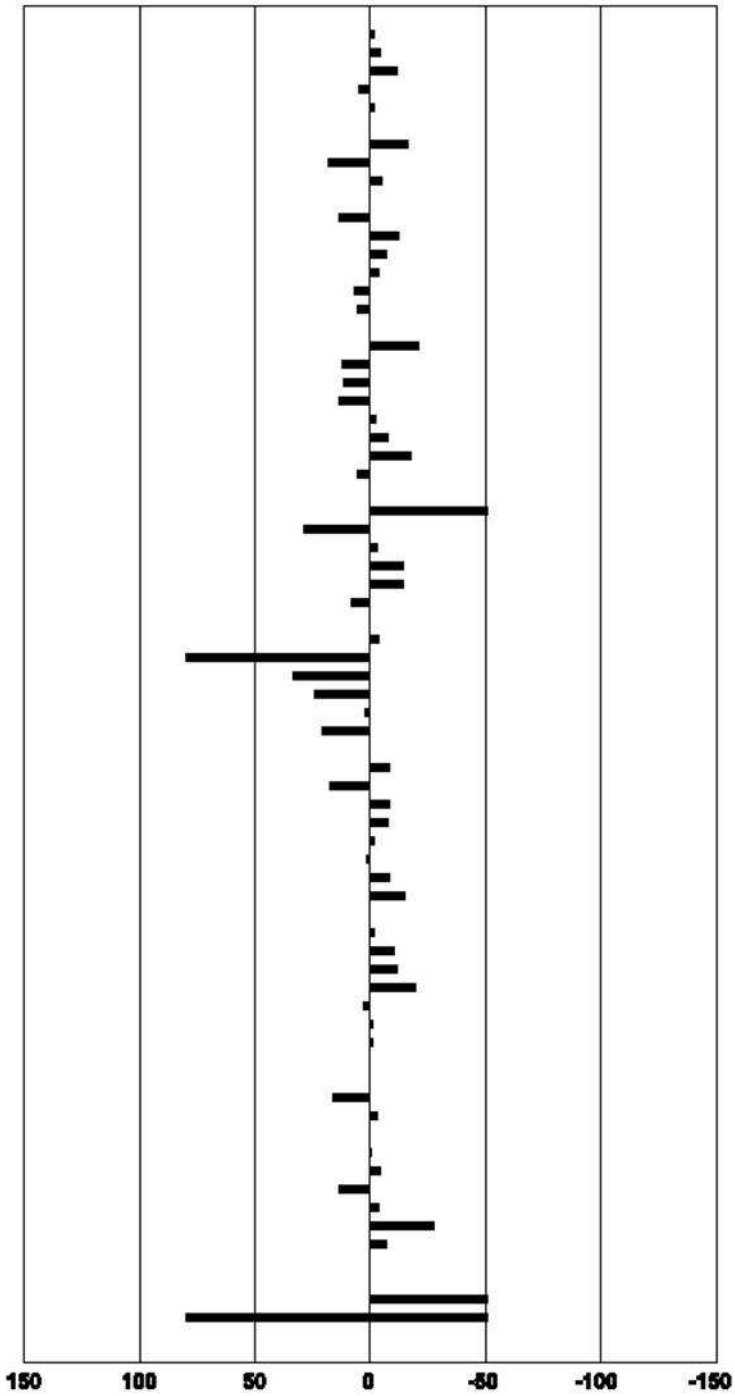



**Supplementary Table 2.** *In vitro* antiproliferative activity after 48 h of 7-azaindirubin (**10**) at $10^{-5}$ M against 57 NCI cancer cell lines




**Supplementary Table 3:** *In vitro* antiproliferative activity after 48 h of 7-azaindirubin-3'-oxime (**12**) at $10^{-5}$ M against 57 NCI cancer cell lines

| Panel/Cell Line | Growth Percent |
|---|---|
| Non-Small Cell Lung Cancer | |
| A549/ATCC | 101.06 |
| EKVX | 98.39 |
| HOP-62 | 91.11 |
| HOP-92 | 107.71 |
| NCI-H226 | 101.61 |
| NCI-H23 | 103.07 |
| NCI-H322M | 96.47 |
| NCI-H460 | 120.96 |
| NCI-H522 | 98.03 |
| Colon Cancer | |
| COLO 205 | 116.61 |
| HCT-116 | 90.43 |
| HCT-15 | 96.05 |
| HT29 | 99.45 |
| KM12 | 109.86 |
| SW-620 | 108.64 |
| Breast Cancer | |
| BT-549 | 81.80 |
| HS 578T | 114.96 |
| MCF7 | 114.39 |
| MDA-MB-231/ATCC | 116.57 |
| MDA-MB-435 | 100.63 |
| MDA-MB-468 | 95.51 |
| NCI/ADR-RES | 95.54 |
| T-47D | 108.89 |
| Ovarian Cancer | |
| IGROV1 | 52.16 |
| OVCAR-3 | 132.01 |
| OVCAR-4 | 99.61 |
| OVCAR-5 | 86.71 |
| OVCAR-8 | 88.79 |
| SK-OV-3 | 110.96 |
| Leukemia | |
| CCRF-CEM | 99.37 |
| HL-60(TB) | 182.86 |
| K-562 | 136.57 |
| MOLT-4 | 127.07 |
| RPMI-8226 | 105.20 |
| SR | 123.56 |
| Renal Cancer | |
| 786-0 | 94.46 |
| A498 | 120.49 |
| ACHN | 94.56 |
| CAKI-1 | 95.53 |
| RXF 393 | 101.15 |
| SN12C | 104.78 |
| TK-10 | 94.36 |
| UO-31 | 88.30 |
| Melanoma | |
| LOX IMVI | 101.46 |
| M14 | 92.67 |
| MALME-3M | 91.46 |
| SK-MEL-2 | 93.47 |
| SK-MEL-28 | 105.94 |
| SK-MEL-5 | 102.06 |
| UACC-257 | 102.20 |
| UACC-62 | 103.02 |
| Prostate Cancer | |
| DU-145 | 119.20 |
| PC-3 | 100.09 |
| CNS Cancer | |
| SF-268 | 102.77 |
| SF-295 | 98.90 |
| SF-539 | 116.61 |
| SNB-19 | 99.41 |
| SNB-75 | 75.52 |
| U251 | 95.82 |
| Mean | 103.04 |
| Delta | 50.86 |
| Range | 130.70 |




**Supplementary Table 4:** *In vitro* antiproliferative activity after 48 h of 7,7'-diaza-1,1'-dimethylisoindigo (**7**) at $10^{-5}$ M against 57 NCI cancer cell lines

| | | | Log10 Concentration | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time | | Mean Optical Densities | | | | | Percent Growth | | | | | | | |
| Panel/Cell Line | Zero | Ctrl | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | -8.0 | -7.0 | -6.0 | -5.0 | -4.0 | GI50 | TGI | LC50 |
| Leukemia | | | | | | | | | | | | | | | |
| CCRF-CEM | 0.183 | 0.633 | 0.605 | 0.526 | 0.552 | 0.348 | 0.345 | 94 | 76 | 82 | 37 | 36 | 5.07E-6 | > 1.00E-4 | > 1.00E-4 |
| HL-60(TB) | 0.344 | 1.785 | 1.832 | 1.771 | 1.490 | 0.898 | 0.447 | 103 | 99 | 80 | 38 | 7 | 5.23E-6 | > 1.00E-4 | > 1.00E-4 |
| K-562 | 0.084 | 1.101 | 0.982 | 0.854 | 0.757 | 0.476 | 0.339 | 88 | 76 | 66 | 39 | 25 | 3.84E-6 | > 1.00E-4 | > 1.00E-4 |
| MOLT-4 | 0.217 | 1.569 | 1.466 | 1.253 | 1.169 | 0.590 | 0.458 | 92 | 77 | 70 | 28 | 18 | 2.99E-6 | > 1.00E-4 | > 1.00E-4 |
| RPMI-8226 | 0.400 | 0.811 | 0.789 | 0.730 | 0.681 | 0.408 | 0.398 | 95 | 80 | 68 | 2 | -1 | 1.89E-6 | 6.25E-5 | > 1.00E-4 |
| SR | 0.217 | 0.575 | 0.609 | 0.537 | 0.742 | 0.322 | 0.309 | 109 | 89 | 147 | 29 | 26 | 6.65E-6 | > 1.00E-4 | > 1.00E-4 |
| Non-Small Cell Lung Cancer | | | | | | | | | | | | | | | |
| A549/ATCC | 0.230 | 0.781 | 0.791 | 0.752 | 0.732 | 0.315 | 0.300 | 102 | 95 | 91 | 15 | 13 | 3.50E-6 | > 1.00E-4 | > 1.00E-4 |
| HOP-62 | 0.452 | 0.897 | 0.907 | 0.897 | 0.919 | 0.733 | 0.782 | 102 | 100 | 105 | 63 | 74 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| HOP-92 | 0.499 | 0.858 | 0.866 | 0.790 | 0.723 | 0.629 | 0.656 | 102 | 81 | 62 | 36 | 44 | 2.97E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H226 | 1.072 | 1.573 | 1.503 | 1.492 | 1.339 | 1.022 | 1.136 | 86 | 84 | 53 | -5 | 13 | 1.14E-6 | . | > 1.00E-4 |
| NCI-H23 | 0.495 | 1.266 | 1.238 | 1.190 | 1.138 | 0.916 | 0.926 | 96 | 90 | 83 | 55 | 56 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| NCI-H322M | 1.004 | 1.726 | 1.691 | 1.596 | 1.410 | 1.063 | 1.089 | 95 | 82 | 56 | 8 | 12 | 1.35E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H460 | 0.191 | 1.548 | 1.690 | 1.617 | 1.509 | 0.753 | 0.627 | 110 | 105 | 97 | 41 | 32 | 7.02E-6 | > 1.00E-4 | > 1.00E-4 |
| NCI-H522 | 0.483 | 1.625 | 1.626 | 1.619 | 1.617 | 1.177 | 1.054 | 100 | 99 | 99 | 61 | 50 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| Colon Cancer | | | | | | | | | | | | | | | |
| COLO 205 | 0.230 | 1.161 | 1.125 | 1.042 | 1.014 | 0.775 | 0.440 | 96 | 87 | 84 | 59 | 23 | 1.73E-5 | > 1.00E-4 | > 1.00E-4 |
| HCC-2998 | 0.512 | 1.001 | 0.968 | 0.938 | 0.944 | 0.702 | 0.699 | 93 | 87 | 88 | 39 | 38 | 5.96E-6 | > 1.00E-4 | > 1.00E-4 |
| HCT-116 | 0.133 | 0.788 | 0.846 | 0.834 | 0.840 | 0.434 | 0.461 | 109 | 107 | 108 | 46 | 50 | . | > 1.00E-4 | > 1.00E-4 |
| HCT-15 | 0.215 | 1.362 | 1.307 | 1.319 | 1.127 | 0.528 | 0.494 | 95 | 96 | 80 | 27 | 24 | 3.67E-6 | > 1.00E-4 | > 1.00E-4 |
| HT29 | 0.168 | 1.086 | 1.077 | 1.066 | 1.072 | 0.908 | 0.612 | 99 | 98 | 99 | 81 | 48 | 8.88E-5 | > 1.00E-4 | > 1.00E-4 |
| KM12 | 0.321 | 1.325 | 1.274 | 1.238 | 0.770 | 0.383 | 0.379 | 95 | 91 | 45 | 6 | 6 | 7.70E-7 | > 1.00E-4 | > 1.00E-4 |
| SW-620 | 0.175 | 0.907 | 0.900 | 0.862 | 0.894 | 0.489 | 0.457 | 99 | 94 | 98 | 43 | 39 | 7.45E-6 | > 1.00E-4 | > 1.00E-4 |
| CNS Cancer | | | | | | | | | | | | | | | |
| SF-268 | 0.337 | 1.060 | 1.061 | 1.053 | 0.998 | 0.724 | 0.727 | 100 | 99 | 91 | 54 | 54 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SF-539 | 0.596 | 1.742 | 1.620 | 1.639 | 1.594 | 1.282 | 1.189 | 89 | 91 | 87 | 60 | 52 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SNB-19 | 0.574 | 1.606 | 1.548 | 1.572 | 1.545 | 1.377 | 1.436 | 94 | 97 | 94 | 78 | 83 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SNB-75 | 0.515 | 0.943 | 0.876 | 0.899 | 0.881 | 0.575 | 0.598 | 84 | 90 | 86 | 14 | 19 | 3.13E-6 | > 1.00E-4 | > 1.00E-4 |
| U251 | 0.286 | 1.270 | 1.238 | 1.243 | 1.159 | 0.829 | 0.952 | 97 | 97 | 89 | 55 | 68 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| Melanoma | | | | | | | | | | | | | | | |
| LOX IMVI | 0.322 | 1.824 | 1.797 | 1.778 | 1.608 | 0.859 | 0.769 | 98 | 97 | 86 | 36 | 30 | 5.17E-6 | > 1.00E-4 | > 1.00E-4 |
| MALME-3M | 0.771 | 1.405 | 1.370 | 1.334 | 1.348 | 0.854 | 0.446 | 94 | 89 | 91 | 13 | -42 | 3.36E-6 | 1.72E-5 | > 1.00E-4 |
| M14 | 0.404 | 1.204 | 1.164 | 1.166 | 1.095 | 0.772 | 0.574 | 95 | 95 | 86 | 46 | 21 | 7.95E-6 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-28 | 0.337 | 1.003 | 0.924 | 0.916 | 0.915 | 0.695 | 0.647 | 88 | 87 | 87 | 54 | 47 | 3.28E-5 | > 1.00E-4 | > 1.00E-4 |
| SK-MEL-5 | 0.696 | 2.331 | 2.390 | 2.303 | 2.238 | 1.498 | 1.190 | 104 | 98 | 94 | 49 | 30 | 9.53E-6 | > 1.00E-4 | > 1.00E-4 |
| UACC-257 | 0.668 | 1.141 | 1.132 | 1.106 | 1.210 | 1.042 | 0.953 | 98 | 92 | 114 | 79 | 60 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| UACC-62 | 0.681 | 2.227 | 2.136 | 2.035 | 1.869 | 1.104 | 1.074 | 94 | 88 | 77 | 27 | 25 | 3.49E-6 | > 1.00E-4 | > 1.00E-4 |
| Ovarian Cancer | | | | | | | | | | | | | | | |
| IGROV1 | 0.274 | 0.988 | 1.001 | 0.876 | 0.703 | 0.366 | 0.363 | 102 | 84 | 60 | 13 | 12 | 1.64E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-3 | 0.349 | 1.041 | 1.034 | 0.990 | 0.906 | 0.703 | 0.700 | 99 | 93 | 80 | 51 | 51 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-4 | 0.427 | 0.960 | 0.929 | 0.943 | 0.939 | 0.683 | 0.634 | 94 | 97 | 96 | 48 | 39 | 9.10E-6 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-5 | 0.425 | 0.902 | 0.849 | 0.848 | 0.873 | 0.845 | 0.659 | 89 | 89 | 94 | 88 | 49 | 9.39E-5 | > 1.00E-4 | > 1.00E-4 |
| OVCAR-8 | 0.173 | 0.710 | 0.599 | 0.629 | 0.624 | 0.442 | 0.454 | 79 | 85 | 84 | 50 | 52 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| SK-OV-3 | 0.541 | 1.303 | 1.304 | 1.250 | 1.067 | 0.508 | 0.602 | 100 | 93 | 69 | -6 | 8 | 1.79E-6 | . | > 1.00E-4 |
| Renal Cancer | | | | | | | | | | | | | | | |
| 786-0 | 0.642 | 1.224 | 1.335 | 1.309 | 1.303 | 0.879 | 0.871 | 119 | 115 | 114 | 41 | 39 | 7.46E-6 | > 1.00E-4 | > 1.00E-4 |
| A498 | 0.739 | 1.253 | 1.132 | 1.178 | 1.062 | 0.832 | 0.960 | 76 | 85 | 63 | 18 | 43 | 1.93E-6 | > 1.00E-4 | > 1.00E-4 |
| ACHN | 0.285 | 1.227 | 1.247 | 1.183 | 0.963 | 0.480 | 0.449 | 102 | 95 | 72 | 21 | 17 | 2.68E-6 | > 1.00E-4 | > 1.00E-4 |
| RXF 393 | 0.437 | 0.606 | 0.593 | 0.596 | 0.531 | 0.399 | 0.449 | 93 | 94 | 55 | -9 | 7 | 1.22E-6 | . | > 1.00E-4 |
| SN12C | 0.426 | 1.447 | 1.378 | 1.338 | 1.249 | 0.801 | 0.790 | 93 | 89 | 81 | 37 | 36 | 4.98E-6 | > 1.00E-4 | > 1.00E-4 |
| TK-10 | 0.424 | 0.895 | 0.898 | 0.915 | 0.852 | 0.657 | 0.491 | 101 | 104 | 91 | 49 | 14 | 9.72E-6 | > 1.00E-4 | > 1.00E-4 |
| UO-31 | 0.626 | 1.363 | 1.255 | 1.216 | 1.080 | 0.649 | 0.707 | 85 | 80 | 62 | 3 | 11 | 1.58E-6 | > 1.00E-4 | > 1.00E-4 |
| Prostate Cancer | | | | | | | | | | | | | | | |
| PC-3 | 0.206 | 0.586 | 0.572 | 0.524 | 0.517 | 0.372 | 0.379 | 96 | 83 | 82 | 44 | 45 | 6.82E-6 | > 1.00E-4 | > 1.00E-4 |
| DU-145 | 0.229 | 0.786 | 0.798 | 0.796 | 0.699 | 0.446 | 0.423 | 102 | 102 | 84 | 39 | 35 | 5.69E-6 | > 1.00E-4 | > 1.00E-4 |
| Breast Cancer | | | | | | | | | | | | | | | |
| MCF7 | 0.200 | 1.078 | 1.058 | 1.000 | 0.957 | 0.660 | 0.558 | 98 | 91 | 86 | 52 | 41 | 1.59E-5 | > 1.00E-4 | > 1.00E-4 |
| NCI/ADR-RES | 0.512 | 1.459 | 1.448 | 1.455 | 1.449 | 1.049 | 1.030 | 99 | 100 | 99 | 57 | 55 | > 1.00E-4 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-231/ATCC | 0.604 | 1.272 | 1.263 | 1.194 | 1.114 | 0.871 | 0.616 | 99 | 88 | 76 | 40 | 2 | 5.28E-6 | > 1.00E-4 | > 1.00E-4 |
| HS 578T | 0.508 | 0.920 | 0.906 | 0.911 | 0.828 | 0.527 | 0.636 | 97 | 98 | 78 | 4 | 31 | 2.39E-6 | > 1.00E-4 | > 1.00E-4 |
| BT-549 | 0.833 | 1.317 | 1.266 | 1.172 | 1.167 | 1.042 | 0.978 | 89 | 70 | 69 | 43 | 30 | 5.41E-6 | > 1.00E-4 | > 1.00E-4 |
| T-47D | 0.485 | 1.075 | 1.074 | 0.992 | 0.870 | 0.578 | 0.609 | 100 | 86 | 65 | 16 | 21 | 2.03E-6 | > 1.00E-4 | > 1.00E-4 |
| MDA-MB-468 | 0.575 | 1.217 | 1.068 | 1.060 | 1.087 | 0.688 | 0.656 | 77 | 75 | 80 | 18 | 13 | 3.01E-6 | > 1.00E-4 | > 1.00E-4 |

**Supplementary Table 5:** *In vitro* antiproliferative activity of 7-azaindirubin-3'-oxime (**12**) against 57 NCI cancer cell lines

Figure S1. $^{1}$H NMR spectrum of 7-azaindirubin (**10**)

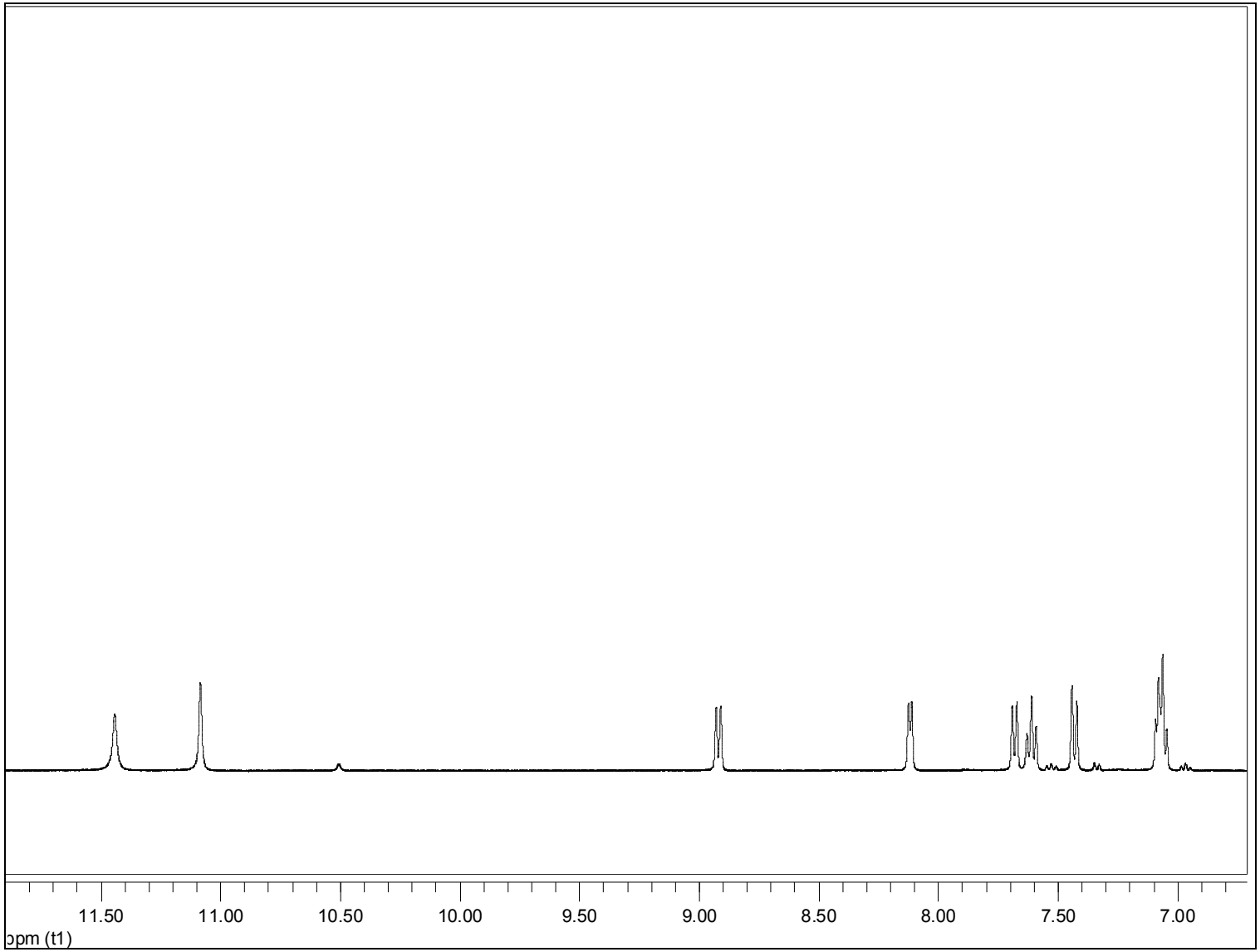


Figure S2. $^{1}$H NMR spectrum of 7-aza-N-methylindirubin (**11**)

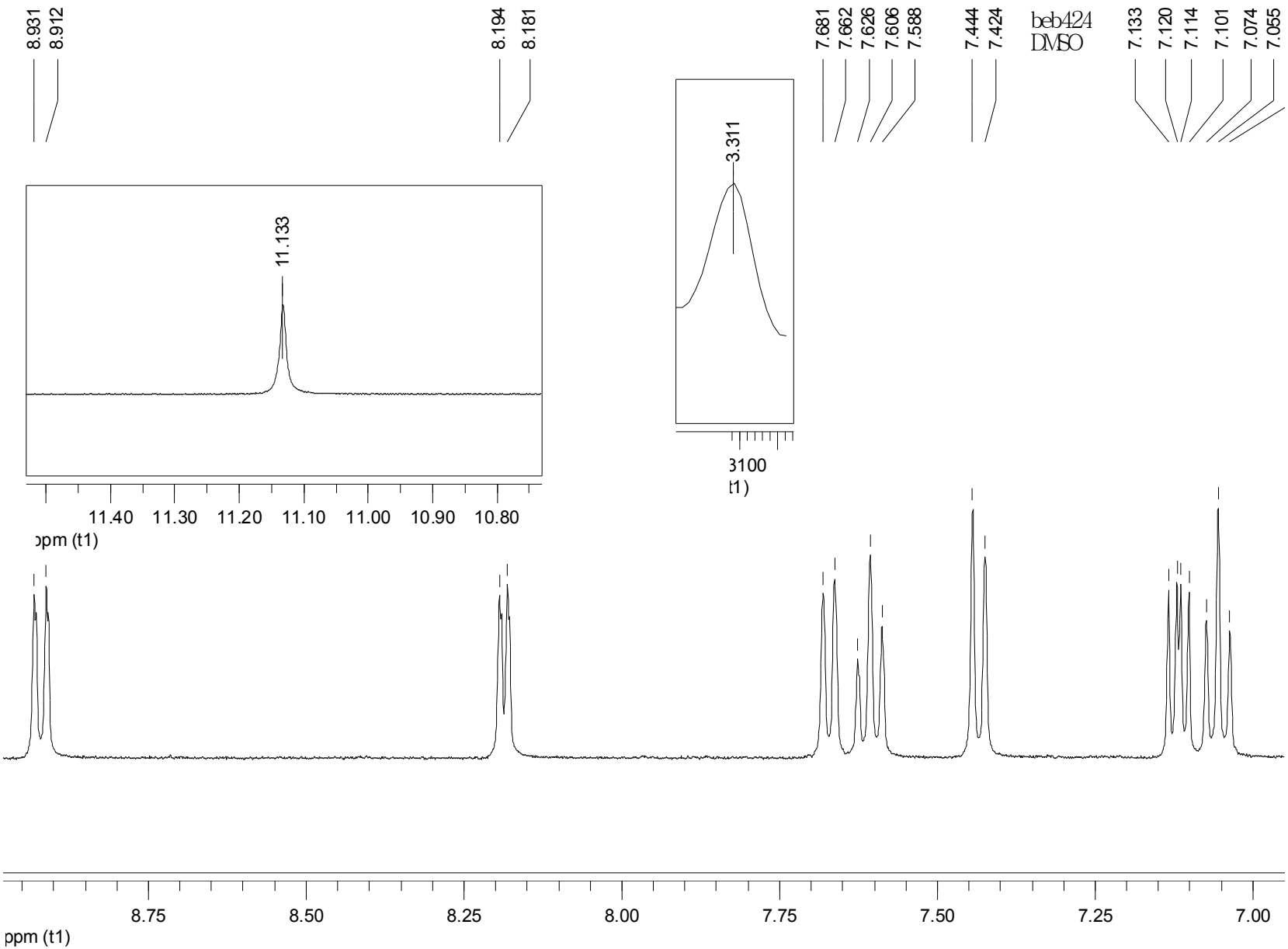


Figure S3. $^{1}$H NMR spectrum of 7-azaindirubin-3'-oxime (**12**)

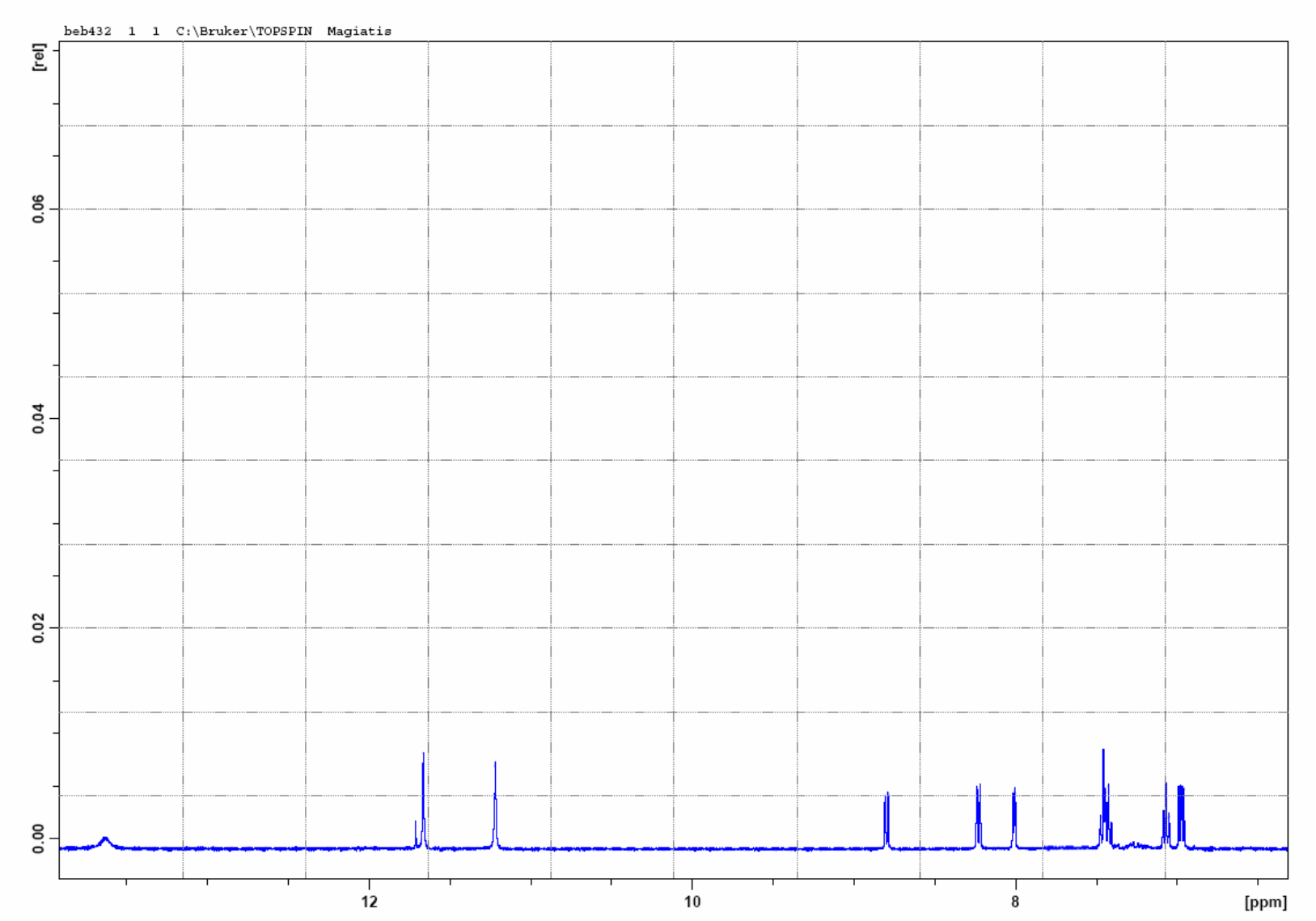


Figure S4. $^{1}$H NMR spectrum of 7-aza-N-methylindirubin-3'-oxime (**13**)

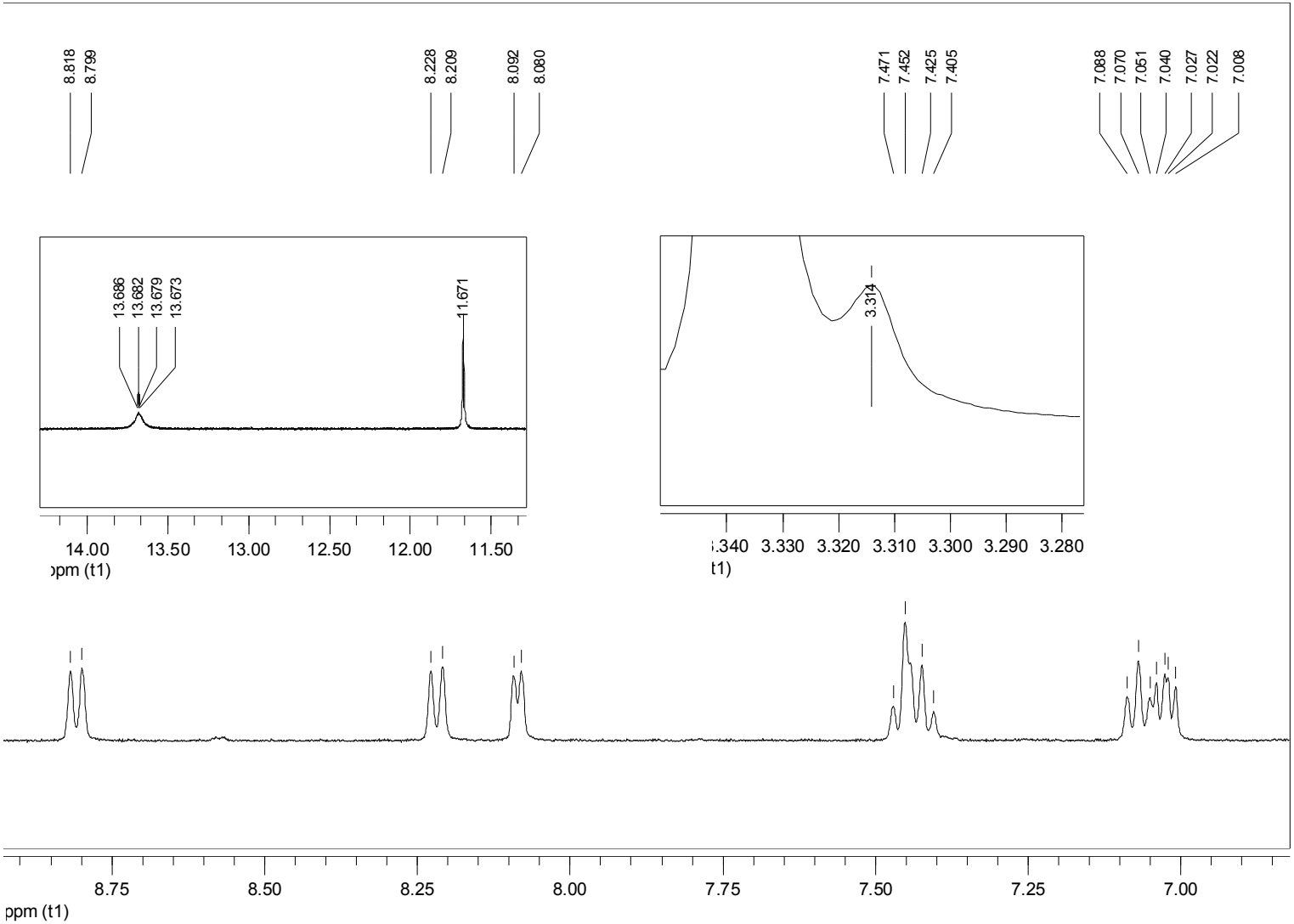


Figure S5. $^{13}C$ NMR spectrum of 7-aza-N-methylindirubin-3'-oxime (**13**)

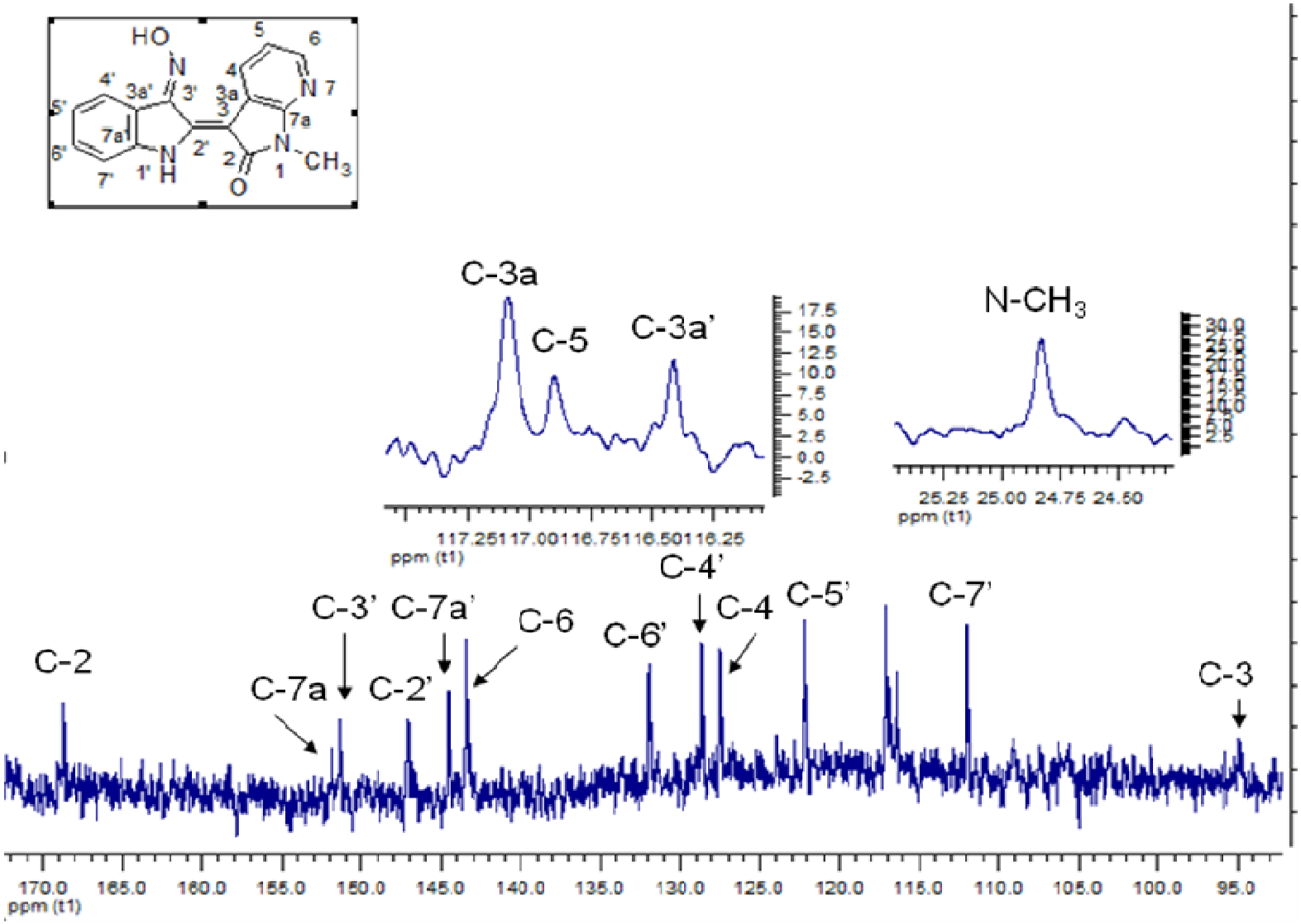
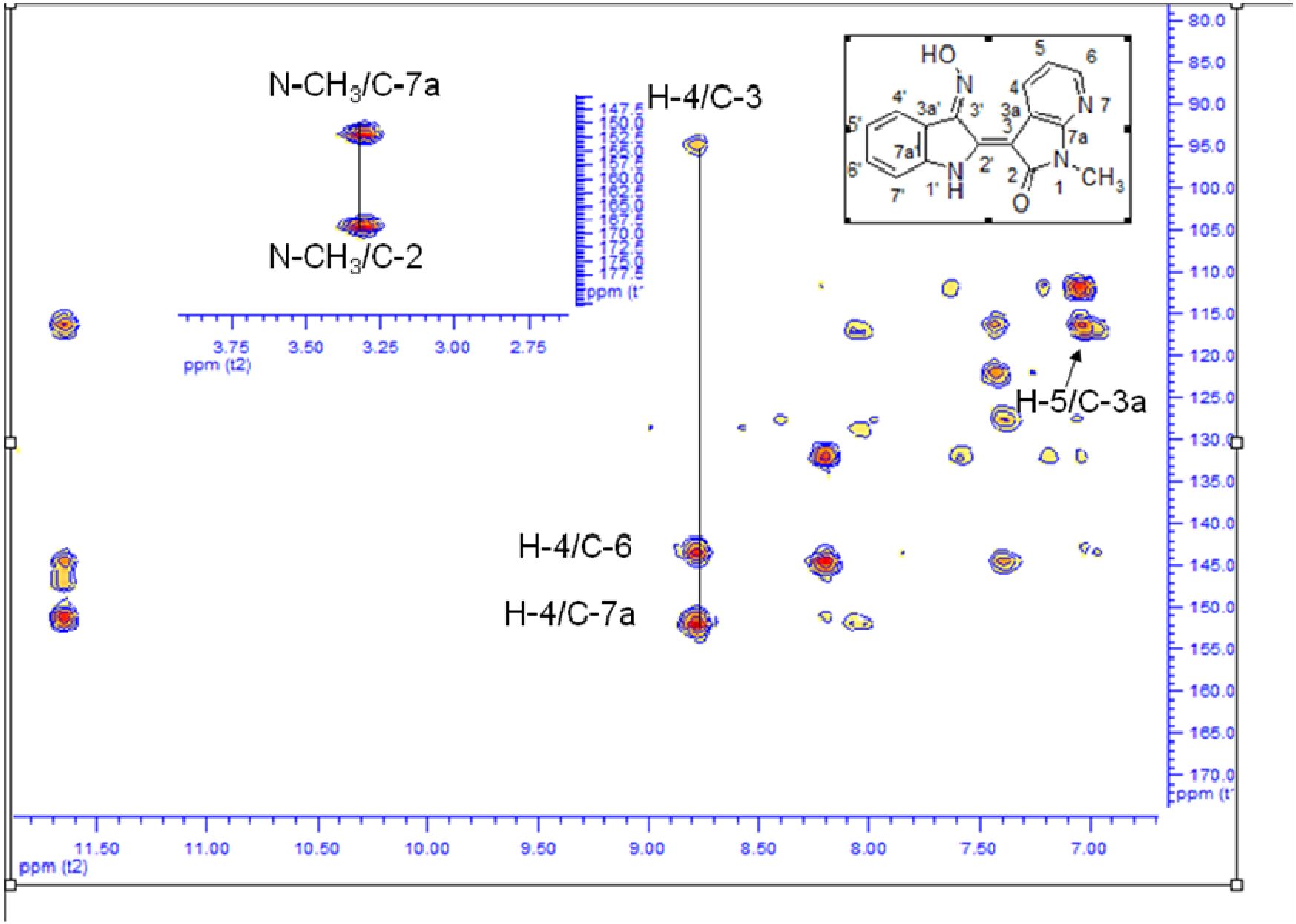



Figure S6. HMBC spectrum of 7-aza-N-methylindirubin-3'-oxime (**13**)