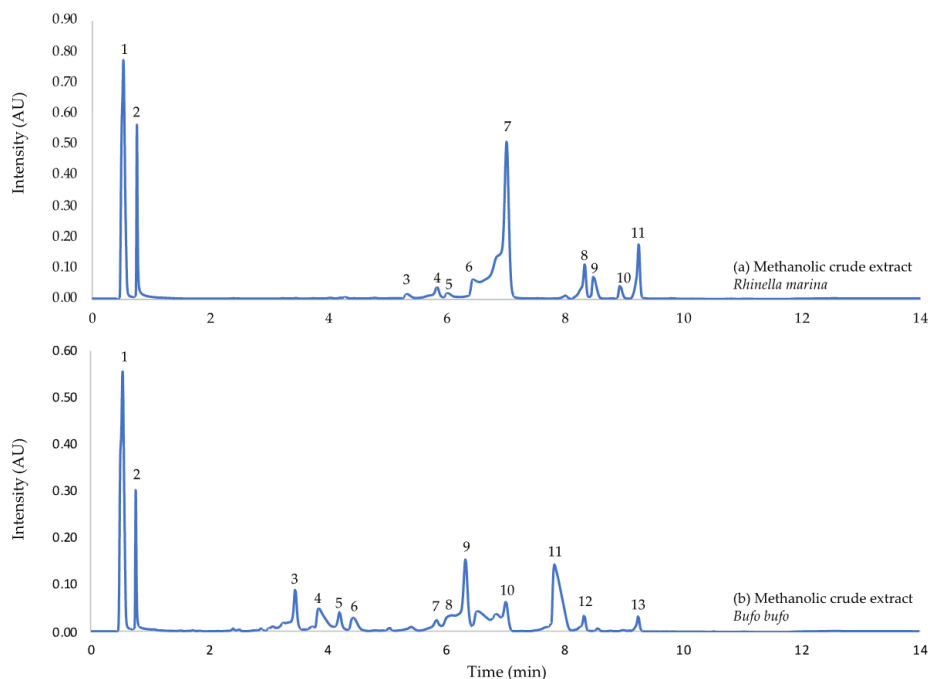




**Figure S1.** Chemical profiles of *Bufo bufo* and *Rhinella marina* crude extracts. UHPLC-UV/MS analyses of: (a) Methanolic crude extract of *Rhinella marina* venom – 1: dehydrobufotenin ( $[M+H]^+ = 203.2$  m/z); 2: suberoyl arginine ( $[M+H]^+ = 331.2$  m/z); 3: marinobufagin-3-pimeloyl-arginine ( $[M+H]^+ = 699.7$  m/z); 4: telocinobufagin ( $[M+H]^+ = 403.4$  m/z); 5: telocinobufotoxin ( $[M+H]^+ = 715.7$  m/z); 6: marinobufotoxin ( $[M+H]^+ = 713.7$  m/z); 7: marinobufagenin ( $[M+H]^+ = 401.4$  m/z); 8 : bufalin ( $[M+H]^+ = 387.4$  m/z); 9: bufalitoxin ( $[M+H]^+ = 699.7$  m/z); 10: resibufotoxin ( $[M+H]^+ = 697.7$  m/z); 11: resibufogenin ( $[M+H]^+ = 385.3$  m/z). (b) Methanolic crude extract of *Bufo bufo* venom – 1: dehydrobufotenin ( $[M+H]^+ = 203.2$  m/z); 2: suberoyl arginine ( $[M+H]^+ = 331.2$  m/z); 3: arenobufagenin ( $[M+H]^+ = 417.4$  m/z); 4: gamabufotalin-3-pelargonate-arginine ( $[M+H]^+ = 729.7$  m/z); 5: unknown identity; 6: bufalin-3-sebacate-arginine ( $[M+H]^+ = 727.6$  m/z); 7: telocinobufagin ( $[M+H]^+ = 403.4$  m/z); 8: telocinobufotoxin ( $[M+H]^+ = 715.7$  m/z); 9: bufotalin ( $[M+H]^+ = 445.4$  m/z); 10: marinobufagenin ( $[M+H]^+ = 401.4$  m/z); 11: 3-oxo-resibufogenin ( $[M+H]^+ = 757.7$  m/z); 12: bufalin ( $[M+H]^+ = 387.3$  m/z); 13: resibufogenin ( $[M+H]^+ = 385.3$  m/z).

**Table S1.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in HBL cells.

| HBL                    | 0.001 $\mu\text{g/mL}$ | 0.01 $\mu\text{g/mL}$ | 0.1 $\mu\text{g/mL}$ | 1 $\mu\text{g/mL}$   | 10 $\mu\text{g/mL}$  |
|------------------------|------------------------|-----------------------|----------------------|----------------------|----------------------|
| <b>Bufo bufo</b>       | 95.02 $\pm$ 5.95 *     | 74.09 $\pm$ 5.25 *    | 34.92 $\pm$ 1.70 **  | 11.84 $\pm$ 0.89 *** | 7.57 $\pm$ 0.32 ***  |
| <b>Rhinella marina</b> | 91.89 $\pm$ 4.05 *     | 87.72 $\pm$ 6.93 *    | 35.95 $\pm$ 8.29 *   | 12.12 $\pm$ 0.67 *** | 5.81 $\pm$ 0.32 ***  |
| <b>Fraction 1</b>      | 99.41 $\pm$ 4.55       | 90.76 $\pm$ 1.92 ***  | 42.29 $\pm$ 4.11 *** | 17.62 $\pm$ 3.16 *** | 11.25 $\pm$ 1.67 *** |
| <b>Fraction 2</b>      | 99.58 $\pm$ 3.33 *     | 99.14 $\pm$ 2.76 *    | 91.33 $\pm$ 4.69 **  | 31.04 $\pm$ 2.91 *** | 14.08 $\pm$ 3.12 *** |
| <b>Fraction 3</b>      | 99.73 $\pm$ 1.96       | 95.85 $\pm$ 3.66 *    | 96.51 $\pm$ 3.64 *   | 88.85 $\pm$ 1.69 *** | 5.27 $\pm$ 3.07 ***  |
| <b>Fraction F'</b>     | 99.42 $\pm$ 3.52       | 99.15 $\pm$ 2.39      | 99.18 $\pm$ 2.19     | 99.31 $\pm$ 2.96     | 91.08 $\pm$ 4.76 *   |
| <b>Internal Mix</b>    | 95.90 $\pm$ 3.76 ***   | 38.53 $\pm$ 2.68 ***  | 16.28 $\pm$ 5.70 *** | 13.97 $\pm$ 5.74 *** | 13.97 $\pm$ 3.75 *** |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S2.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in HBL cells.

| HBL           | 0.00001 $\mu$ M      | 0.0001 $\mu$ M       | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| MBG           | 99.92 $\pm$ 6.31     | 99.61 $\pm$ 5.52     | 99.99 $\pm$ 4.35     | 99.37 $\pm$ 3.40     | 31.11 $\pm$ 2.90 *** | 13.73 $\pm$ 5.73 *** | 10.76 $\pm$ 5.89 *** |
| RBG           | 99.97 $\pm$ 7.12     | 98.91 $\pm$ 5.67     | 95.71 $\pm$ 6.66     | 82.64 $\pm$ 5.43 *** | 28.05 $\pm$ 3.33 *** | 18.90 $\pm$ 4.67 *** | 19.78 $\pm$ 4.67 *** |
| Bufalin       | 85.32 $\pm$ 1.86 *** | 46.05 $\pm$ 5.10 *** | 26.15 $\pm$ 7.49 *** | 19.67 $\pm$ 5.60 *** | 14.12 $\pm$ 8.46 *** | 11.26 $\pm$ 5.76 *** | 11.87 $\pm$ 5.09 *** |
| TBG           | 99.89 $\pm$ 2.65     | 99.51 $\pm$ 5.23     | 81.84 $\pm$ 6.06 *** | 23.84 $\pm$ 4.30 *** | 17.20 $\pm$ 4.27 *** | 14.66 $\pm$ 5.73 *** | 13.77 $\pm$ 4.73 *** |
| Bufalin + MBG | 91.24 $\pm$ 5.44 *   | 93.92 $\pm$ 2.27 *   | 34.96 $\pm$ 9.16 *** | 11.83 $\pm$ 7.40 *** | 10.10 $\pm$ 6.04 *** | /                    | /                    |
| Bufalin + RBG | 90.68 $\pm$ 2.83 *** | 86.75 $\pm$ 2.28 *** | 36.70 $\pm$ 5.63 *** | 7.34 $\pm$ 3.63 ***  | 3.99 $\pm$ 1.82 ***  | /                    | /                    |
| Bufalin + TBG | 92.32 $\pm$ 4.31 **  | 49.17 $\pm$ 8.27 *** | 16.29 $\pm$ 9.36 *** | 7.01 $\pm$ 3.96 ***  | 4.76 $\pm$ 1.82 ***  | /                    | /                    |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S3.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in HBL-R cells.

| HBL-R           | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|-----------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Bufo bufo       | 98.03 $\pm$ 4.87 *   | 66.57 $\pm$ 4.76 **  | 29.61 $\pm$ 1.22 *** | 18.69 $\pm$ 0.98 *** | 16.18 $\pm$ 0.91 *** |
| Rhinella marina | 99.79 $\pm$ 3.06     | 66.57 $\pm$ 3.78     | 38.22 $\pm$ 2.07 **  | 18.21 $\pm$ 0.66 *** | 14.66 $\pm$ 0.52 *** |
| Fraction 1      | 99.74 $\pm$ 2.79 *   | 99.76 $\pm$ 2.66 *   | 34.06 $\pm$ 2.87 *** | 11.63 $\pm$ 2.67 *** | 6.20 $\pm$ 1.80 ***  |
| Fraction 2      | 99.16 $\pm$ 1.63     | 99.94 $\pm$ 2.40     | 85.38 $\pm$ 4.60 *** | 26.15 $\pm$ 5.80 *** | 7.48 $\pm$ 5.40 ***  |
| Fraction 3      | 99.73 $\pm$ 1.96     | 95.85 $\pm$ 3.66     | 96.51 $\pm$ 3.64     | 88.58 $\pm$ 1.69     | 5.27 $\pm$ 3.07 ***  |
| Fraction F'     | 99.05 $\pm$ 2.79     | 99.27 $\pm$ 2.66     | 99.73 $\pm$ 2.87     | 99.06 $\pm$ 2.67     | 98.67 $\pm$ 1.80     |
| Internal Mix    | 82.80 $\pm$ 2.68 *** | 37.00 $\pm$ 8.07 *** | 23.48 $\pm$ 4.97 *** | 16.11 $\pm$ 5.97 *** | 11.14 $\pm$ 3.46 *** |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S4.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in HBL-R cells.

| HBL-R         | 0.00001 $\mu$ M      | 0.0001 $\mu$ M       | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M           | 1 $\mu$ M            | 10 $\mu$ M           |
|---------------|----------------------|----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|
| MBG           | 99.41 $\pm$ 3.17     | 99.47 $\pm$ 4.80     | 93.79 $\pm$ 7.05     | 80.07 $\pm$ 6.93 *** | 34.22 $\pm$ 6.21 ***  | 19.89 $\pm$ 1.91 *** | 17.76 $\pm$ 7.79 *** |
| RBG           | 99.59 $\pm$ 2.63     | 99.52 $\pm$ 9.22     | 97.88 $\pm$ 4.58 *   | 73.86 $\pm$ 3.19 *** | 37.70 $\pm$ 9.45 ***  | 31.80 $\pm$ 7.73 *** | 30.59 $\pm$ 9.19 *** |
| Bufalin       | 63.41 $\pm$ 1.69 *** | 34.28 $\pm$ 2.91 *** | 23.67 $\pm$ 4.46 *** | 18.08 $\pm$ 6.22 *** | 12.82 $\pm$ 3.89 ***  | 12.93 $\pm$ 7.99 *** | 10.85 $\pm$ 7.84 *** |
| TBG           | 99.63 $\pm$ 4.20     | 95.42 $\pm$ 4.74     | 53.64 $\pm$ 4.11 *** | 28.45 $\pm$ 5.40 *** | 19.90 $\pm$ 10.46 *** | 18.41 $\pm$ 7.89 *** | 16.69 $\pm$ 8.40 *** |
| Bufalin + MBG | 67.61 $\pm$ 9.72 *** | 66.90 $\pm$ 7.24 *** | 41.47 $\pm$ 9.72 *** | 17.33 $\pm$ 9.51 *** | 9.72 $\pm$ 5.35 ***   | /                    | /                    |
| Bufalin + RBG | 82.28 $\pm$ 3.13 *** | 79.48 $\pm$ 2.46 *** | 38.72 $\pm$ 4.53 *** | 12.68 $\pm$ 6.55 *** | 6.79 $\pm$ 3.69 ***   | /                    | /                    |
| Bufalin + TBG | 93.55 $\pm$ 6.94     | 92.37 $\pm$ 3.25     | 37.42 $\pm$ 3.00 *** | 12.05 $\pm$ 5.88 *** | 7.51 $\pm$ 3.80 ***   | /                    | /                    |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

**Table S5.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in MM074 cells.

| MM074           | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|-----------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Bufo bufo       | 98.79 $\pm$ 3.74     | 96.16 $\pm$ 4.88     | 40.51 $\pm$ 2.10 **  | 25.97 $\pm$ 1.36 **  | 23.64 $\pm$ 1.21 **  |
| Rhinella marina | 97.12 $\pm$ 6.71     | 95.06 $\pm$ 5.30     | 45.49 $\pm$ 3.26 **  | 24.28 $\pm$ 1.85 **  | 21.11 $\pm$ 1.69 **  |
| Fraction 1      | 99.56 $\pm$ 4.47     | 93.16 $\pm$ 3.19 **  | 44.63 $\pm$ 4.61 *** | 18.18 $\pm$ 6.03 *** | 13.58 $\pm$ 3.22 *** |
| Fraction 2      | 92.27 $\pm$ 4.62 *** | 91.92 $\pm$ 4.18 *** | 90.59 $\pm$ 4.22 *** | 28.44 $\pm$ 4.86 *** | 16.41 $\pm$ 3.96 *** |
| Fraction 3      | 96.76 $\pm$ 4.77 *   | 94.98 $\pm$ 3.82 *   | 93.31 $\pm$ 2.22 *** | 81.59 $\pm$ 5.14 *** | 6.32 $\pm$ 2.72 ***  |

|                     |                 |                  |                  |                  |                  |
|---------------------|-----------------|------------------|------------------|------------------|------------------|
| <b>Fraction F'</b>  | 94.90 ± 2.24 ** | 91.98 ± 3.95 **  | 90.60 ± 3.40 *** | 86.48 ± 3.26 *** | 83.87 ± 3.60 *** |
| <b>Internal Mix</b> | 93.81 ± 9.75    | 57.10 ± 8.49 *** | 35.08 ± 6.99 *** | 33.67 ± 8.01 *** | 31.62 ± 8.64 *** |

Data are presented as mean ± standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S6.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in MM074 cells.

| MM074                | 0.00001 $\mu$ M  | 0.0001 $\mu$ M   | 0.001 $\mu$ M    | 0.01 $\mu$ M     | 0.1 $\mu$ M      | 1 $\mu$ M        | 10 $\mu$ M       |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| <b>MBG</b>           | 96.36 ± 4.35     | 95.43 ± 4.29     | 97.44 ± 1.73     | 95.54 ± 2.08 *   | 52.37 ± 1.24 *** | 27.17 ± 4.34 *** | 25.06 ± 5.76 *** |
| <b>RBG</b>           | 99.34 ± 6.71     | 95.68 ± 4.12     | 98.83 ± 4.55     | 94.95 ± 3.77 *   | 55.89 ± 5.15 *** | 34.00 ± 4.39 *** | 30.74 ± 3.66 *** |
| <b>Bufalin</b>       | 95.57 ± 4.97 *   | 55.88 ± 5.75 *** | 38.05 ± 3.74 *** | 30.43 ± 4.99 *** | 27.19 ± 3.80 *** | 25.98 ± 7.86 *** | 28.11 ± 7.33 *** |
| <b>TBG</b>           | 98.23 ± 9.36     | 99.67 ± 7.89     | 99.68 ± 6.70 *   | 55.40 ± 4.73 *** | 37.79 ± 6.68 *** | 36.56 ± 4.59 *** | 37.84 ± 2.01 *** |
| <b>Bufalin + MBG</b> | 92.64 ± 9.28 *   | 90.16 ± 8.27 *   | 74.54 ± 7.08 *** | 31.07 ± 6.91 *** | 21.85 ± 6.00 *** | /                | /                |
| <b>Bufalin + RBG</b> | 90.47 ± 3.07     | 89.63 ± 8.71     | 44.20 ± 4.14 *** | 21.42 ± 5.62 *** | 17.56 ± 3.12 *** | /                | /                |
| <b>Bufalin + TBG</b> | 59.38 ± 4.37 *** | 46.41 ± 7.78 *** | 36.17 ± 9.30 *** | 20.44 ± 3.87 *** | 16.53 ± 3.35 *** | /                | /                |

Data are presented as mean ± standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

**Table S7.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in MM074-R cells.

| MM074-R                | 0.001 $\mu$ M  | 0.01 $\mu$ M     | 0.1 $\mu$ M      | 1 $\mu$ M        | 10 $\mu$ M       |
|------------------------|----------------|------------------|------------------|------------------|------------------|
| <b>Bufo bufo</b>       | 98.15 ± 4.74   | 71.56 ± 3.65     | 64.06 ± 2.76 **  | 56.53 ± 1.10 **  | 52.31 ± 1.68 **  |
| <b>Rhinella marina</b> | 99.54 ± 4.06   | 96.57 ± 4.95     | 67.51 ± 2.79 **  | 58.16 ± 1.89 *** | 54.77 ± 2.06 **  |
| <b>Fraction 1</b>      | 93.53 ± 3.86 * | 81.03 ± 4.01 *** | 34.01 ± 3.97 *** | 16.73 ± 4.87 *** | 11.86 ± 3.94 *** |
| <b>Fraction 2</b>      | 99.86 ± 1.08   | 99.91 ± 2.12     | 83.48 ± 2.56 *** | 36.72 ± 2.79 *** | 22.31 ± 2.26 *** |
| <b>Fraction 3</b>      | 99.55 ± 3.71   | 96.15 ± 4.25 *   | 94.80 ± 2.63 *** | 71.80 ± 4.11 *** | 5.13 ± 2.45 ***  |
| <b>Fraction F'</b>     | 99.95 ± 5.10   | 98.37 ± 5.16     | 94.25 ± 6.74 *   | 92.56 ± 6.76 *   | 77.44 ± 7.58 *** |
| <b>Internal Mix</b>    | 99.54 ± 8.03   | 56.73 ± 3.00 *** | 36.90 ± 4.09 *** | 30.18 ± 5.46 *** | 30.39 ± 3.83 *** |

Data are presented as mean ± standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S8.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in MM074-R cells.

| MM074-R              | 0.00001 $\mu$ M  | 0.0001 $\mu$ M   | 0.001 $\mu$ M    | 0.01 $\mu$ M     | 0.1 $\mu$ M      | 1 $\mu$ M        | 10 $\mu$ M       |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| <b>MBG</b>           | 99.54 ± 6.21     | 99.55 ± 10.37    | 99.13 ± 8.47 *   | 95.75 ± 6.75     | 41.72 ± 3.36 *** | 26.01 ± 2.99 *** | 24.8 ± 2.13 ***  |
| <b>RBG</b>           | 92.27 ± 6.52     | 99.54 ± 4.29     | 96.91 ± 2.25     | 92.66 ± 2.46 *   | 47.95 ± 8.50 *** | 37.15 ± 4.09 *** | 42.48 ± 4.78 *** |
| <b>Bufalin</b>       | 83.55 ± 1.95 *** | 45.94 ± 3.27 *** | 36.93 ± 4.60 *** | 29.11 ± 2.74 *** | 65.80 ± 2.74 *** | 26.32 ± 3.11 *** | 26.18 ± 5.72 *** |
| <b>TBG</b>           | 99.72 ± 5.43     | 99.88 ± 4.77     | 78.85 ± 5.41 *** | 42.77 ± 4.93 *** | 30.02 ± 3.40 *** | 27.87 ± 4.86 *** | 26.85 ± 3.47 *** |
| <b>Bufalin + MBG</b> | 97.32 ± 5.45 *   | 89.59 ± 7.31 *   | 49.81 ± 8.15 *** | 20.76 ± 6.07 *** | 17.31 ± 5.75 *** | /                | /                |
| <b>Bufalin + RBG</b> | 99.94 ± 7.97     | 99.05 ± 5.05     | 61.84 ± 5.45 *** | 43.52 ± 3.39 *** | 32.02 ± 9.56 *** | /                | /                |
| <b>Bufalin + TBG</b> | 99.77 ± 6.82     | 62.23 ± 9.56 *** | 45.93 ± 4.09 *** | 38.11 ± 2.70 *** | 32.34 ± 4.55 *** | /                | /                |

Data are presented as mean ± standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

**Table S9.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in MM161 cells.

| MM161                  | 0.001 $\mu$ M      | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|------------------------|--------------------|----------------------|----------------------|----------------------|----------------------|
| <b>Bufo bufo</b>       | 99.07 $\pm$ 3.99   | 98.01 $\pm$ 2.90     | 60.98 $\pm$ 2.51 **  | 38.11 $\pm$ 4.31 **  | 29.14 $\pm$ 1.99 **  |
| <b>Rhinella marina</b> | 92.71 $\pm$ 3.18   | 96.57 $\pm$ 4.65     | 63.45 $\pm$ 2.85 **  | 33.88 $\pm$ 1.62 **  | 26.89 $\pm$ 2.27 **  |
| <b>Fraction 1</b>      | 95.66 $\pm$ 2.39 * | 96.47 $\pm$ 3.54     | 70.91 $\pm$ 4.37 *** | 30.23 $\pm$ 5.02 *** | 15.00 $\pm$ 5.39 *** |
| <b>Fraction 2</b>      | 99.25 $\pm$ 4.29   | 99.49 $\pm$ 3.51     | 94.60 $\pm$ 3.42     | 54.11 $\pm$ 5.47 *** | 18.71 $\pm$ 4.62 *** |
| <b>Fraction 3</b>      | 99.65 $\pm$ 4.91   | 96.79 $\pm$ 3.41 *   | 95.26 $\pm$ 4.31 *** | 85.20 $\pm$ 6.72 *** | 6.18 $\pm$ 2.74 ***  |
| <b>Fraction F'</b>     | 99.24 $\pm$ 3.04   | 99.62 $\pm$ 1.55     | 97.60 $\pm$ 1.58 *   | 96.69 $\pm$ 1.12 *** | 91.16 $\pm$ 2.93 *** |
| <b>Internal Mix</b>    | 99.13 $\pm$ 5.41   | 67.42 $\pm$ 3.73 *** | 33.93 $\pm$ 4.92 *** | 20.86 $\pm$ 5.87 *** | 11.92 $\pm$ 2.59 *** |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S10.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in MM161 cells.

| MM161                | 0.00001 $\mu$ M    | 0.0001 $\mu$ M       | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|----------------------|--------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| <b>MBG</b>           | 99.33 $\pm$ 5.62   | 98.20 $\pm$ 5.09     | 99.85 $\pm$ 7.36     | 99.33 $\pm$ 2.98     | 68.51 $\pm$ 6.43 *** | 25.72 $\pm$ 6.43 *** | 15.06 $\pm$ 4.84 *** |
| <b>RBG</b>           | 99.20 $\pm$ 5.38   | 99.20 $\pm$ 5.84     | 95.99 $\pm$ 3.74 *   | 95.67 $\pm$ 3.63 *   | 60.68 $\pm$ 4.81 *** | 33.83 $\pm$ 6.91 *** | 25.08 $\pm$ 5.87 *** |
| <b>Bufalin</b>       | 99.81 $\pm$ 3.97   | 80.5 $\pm$ 4.26 ***  | 44.14 $\pm$ 5.67 *** | 28.98 $\pm$ 4.70 *** | 20.57 $\pm$ 5.67 *** | 15.39 $\pm$ 6.00 *** | 13.22 $\pm$ 3.82 *** |
| <b>TBG</b>           | 99.88 $\pm$ 2.59   | 98.69 $\pm$ 1.09     | 93.12 $\pm$ 1.59 *   | 48.15 $\pm$ 6.14 *** | 29.35 $\pm$ 6.95 *** | 21.20 $\pm$ 9.41 *** | 20.23 $\pm$ 9.57 *** |
| <b>Bufalin + MBG</b> | 95.41 $\pm$ 4.22 * | 95.27 $\pm$ 3.98 *   | 75.21 $\pm$ 5.66 *** | 42.30 $\pm$ 5.44 *** | 30.01 $\pm$ 9.99 *** | /                    | /                    |
| <b>Bufalin + RBG</b> | 95.50 $\pm$ 5.76   | 89.91 $\pm$ 4.86 **  | 52.22 $\pm$ 8.78 *** | 25.52 $\pm$ 4.52 *** | 19.25 $\pm$ 7.12 *** | /                    | /                    |
| <b>Bufalin + TBG</b> | 88.07 $\pm$ 7.41   | 83.63 $\pm$ 7.37 *** | 50.25 $\pm$ 2.96 *** | 27.07 $\pm$ 3.14 *** | 18.92 $\pm$ 7.62 *** | /                    | /                    |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S11.** Percentage of cell viability for each tested concentration and for each crude extract or fraction in MM161-R cells.

| MM161-R                | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M          | 1 $\mu$ M            | 10 $\mu$ M           |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| <b>Bufo bufo</b>       | 84.05 $\pm$ 3.19 *** | 63.64 $\pm$ 7.39 *** | 37.05 $\pm$ 7.65 *** | 32.99 $\pm$ 7.44 *** | 19.55 $\pm$ 3.05 *** |
| <b>Rhinella marina</b> | 96.85 $\pm$ 5.38     | 88.33 $\pm$ 4.81 **  | 55.48 $\pm$ 2.09 *** | 42.40 $\pm$ 2.61 *** | 32.13 $\pm$ 3.42 *** |
| <b>Fraction 1</b>      | 97.02 $\pm$ 3.91     | 84.50 $\pm$ 2.90 *** | 33.79 $\pm$ 4.45 *** | 15.97 $\pm$ 5.90 *** | 11.40 $\pm$ 4.38 *** |
| <b>Fraction 2</b>      | 91.16 $\pm$ 4.66 *   | 87.28 $\pm$ 7.00 *** | 78.52 $\pm$ 6.79 *** | 27.64 $\pm$ 8.20 *** | 17.41 $\pm$ 6.87 *** |
| <b>Fraction 3</b>      | 99.97 $\pm$ 5.49     | 99.78 $\pm$ 5.56 *   | 97.74 $\pm$ 5.44     | 62.64 $\pm$ 4.14 *** | 10.88 $\pm$ 5.42 *** |
| <b>Fraction F'</b>     | 99.31 $\pm$ 6.70     | 99.01 $\pm$ 5.64     | 99.73 $\pm$ 4.27     | 98.99 $\pm$ 5.46     | 77.24 $\pm$ 5.99 *** |
| <b>Internal Mix</b>    | 96.39 $\pm$ 2.37 *   | 57.99 $\pm$ 4.76 *** | 34.62 $\pm$ 4.68 *** | 25.81 $\pm$ 8.92 *** | 16.01 $\pm$ 7.97 *** |

Data are presented as mean  $\pm$  standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table S12.** Percentage of cell viability for each tested concentration and for each bufadienolide alone or in combination with bufalin in MM161-R cells.

| MM161-R        | 0.00001 $\mu$ M  | 0.0001 $\mu$ M       | 0.001 $\mu$ M        | 0.01 $\mu$ M         | 0.1 $\mu$ M           | 1 $\mu$ M            | 10 $\mu$ M           |
|----------------|------------------|----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|
| <b>MBG</b>     | 96.62 $\pm$ 7.43 | 97.43 $\pm$ 2.45     | 89.76 $\pm$ 3.63 *   | 90.35 $\pm$ 3.43 *   | 49.22 $\pm$ 5.27 ***  | 25.08 $\pm$ 6.40 *** | 17.28 $\pm$ 6.38 *** |
| <b>RBG</b>     | 97.64 $\pm$ 2.48 | 94.92 $\pm$ 4.75     | 96.42 $\pm$ 6.66     | 88.15 $\pm$ 3.74 **  | 50.49 $\pm$ 8.87 ***  | 37.45 $\pm$ 8.56 *** | 30.83 $\pm$ 8.56 *** |
| <b>Bufalin</b> | 93.32 $\pm$ 3.34 | 64.62 $\pm$ 2.59 *** | 39.01 $\pm$ 7.55 *** | 27.06 $\pm$ 6.40 *** | 18.06 $\pm$ 6.26 ***  | 14.96 $\pm$ 8.25 *** | 13.98 $\pm$ 7.42 *** |
| <b>TBG</b>     | 99.30 $\pm$ 4.13 | 95.05 $\pm$ 5.31     | 88.11 $\pm$ 2.14 *** | 42.78 $\pm$ 3.31 *** | 25.56 $\pm$ 10.08 *** | 19.69 $\pm$ 6.12 *** | 17.98 $\pm$ 7.34 *** |

|                          |                 |                     |                      |                     |                     |   |   |
|--------------------------|-----------------|---------------------|----------------------|---------------------|---------------------|---|---|
| <b>Bufalin +<br/>MBG</b> | 92.17 ±<br>4.74 | 95.65 ±<br>10.56    | 61.45 ±<br>10.15 *** | 32.56 ±<br>3.65 *** | 22.82 ± 7.31<br>*** | / | / |
| <b>Bufalin +<br/>RBG</b> | 92.46 ±<br>2.67 | 86.55 ±<br>2.88 *   | 60.18 ± 3.71<br>***  | 26.46 ±<br>6.06 *** | 13.70 ± 8.03<br>*** | / | / |
| <b>Bufalin +<br/>TBG</b> | 96.98 ±<br>2.89 | 76.26 ±<br>6.39 *** | 48.05 ± 4.91<br>***  | 23.43 ±<br>4.91 *** | 14.45 ± 5.77<br>*** | / | / |

Data are presented as mean ± standard deviation. Statistical significance is resulting from Student t tests and is presented as follows: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .