molecules

# High Resolution Mass Profile of Bufadienolides and Peptides Combing with Anti-Tumor Cell Screening and Multivariate Analysis for the Quality Evaluation of Bufonis Venenum

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## Supplementary Materials:

**Table. S1.** Structure of the main compounds in Bufonis Venenum.

| Compound | Skeletons | R | n |
|---|---|---|---|
| bufalin | A1 | $R_1$=$R_3$=H, $R_2$=CH3, $R_5$=OH, $R_4$=$R_6$=$R_7$=$R_8$=$R_9$=H | - |
| gamabufotalin | A1 | $R_1$=$R_3$=$R_4$=$R_7$=$R_8$=$R_9$=H, $R_2$=$CH_3$, $R_5$=OH, $R_6$=α-OH | - |
| bufotalin | A1 | $R_1$=$R_3$=$R_4$=$R_6$=$R_7$=$R_9$=H, $R_2$=$CH_3$, $R_5$=OH, $R_8$=β-OAc | - |
| hellebrigenin | A1 | $R_1$=$R_3$=$R_6$=$R_7$=$R_8$=$R_9$=H, $R_2$=CHO, $R_4$=$R_5$=OH | - |
| telocinobufagin | A1 | $R_1$=$R_3$=$R_6$=$R_7$=$R_8$=$R_9$=H, $R_2$=$CH_3$, $R_4$=$R_5$=OH | - |
| resibufogenin | A2 | $R_1$=$R_3$=$R_4$=$R_5$=$R_6$=$R_7$=$R_8$=H, $R_2$=$CH_3$ | - |
| resibufagin | A2 | $R_1$=$R_3$=$R_4$=$R_5$=$R_6$=$R_7$=$R_8$=H, $R_2$=CHO | - |
| 3β-formyloxylresibufogenin | A2 | $R_1$=HCO, R3=R4=R5=R6=R7=R8=H, R2=CH3 | - |
| 12β-hydroxyresibufaginol | A2 | $R_1$=R3=R4=R5=R6=R8=H, R2=CH3, R7=β-OH | - |
| resibufaginol | A2 | $R_1$=R3=R4=R5=R6=R7=R8=H, R2=CH2OH | - |
| cinobufagin | A2 | $R_1$=$R_3$=$R_4$=$R_5$=$R_6$=$R_7$=H, $R_2$=$CH_3$, $R_8$=β-OAc | - |
| desacetylcinobufagin | A2 | $R_1$=$R_3$=$R_4$=$R_5$=$R_6$=$R_7$=H, $R_2$=$CH_3$, $R_8$=β-OH | - |
| cinobufotalin | A2 | $R_1$=$R_3$=$R_5$=$R_6$=$R_7$=H, $R_2$=$CH_3$, $R_4$=OH, $R_8$=β-OAc | - |
| desacetylcinobufotalin | A2 | $R_1$=$R_3$=$R_5$=$R_6$=$R_7$=H, $R_4$=OH, $R_2$=$CH_3$, $R_8$=β-OH | - |
| arenobufagin | A3 | $R_1$=$R_2$=H | - |
| psi-bufarenogin | A4 | $R_1$=α-OH, R2=H | - |
| bufarenogin | A4 | $R_1$=β-OH, R2=H | - |
| 20S,21-epoxyresibufogenin | A5 | $R_1$=OH | - |
| 20R,21-epoxyresibufogenin | A5 | $R_1$=OH | - |
| 3-O-formyl-20R,21-epoxyresibufogenin | A5 | $R_1$=HCO | - |
| 3-(N-succinyl argininyl) resibufogenin | B | $R_1$= $CH_3$, $R_2$=$R_3$=H | 2 |
| 3-(N-pimeloyl argininyl)bufalin | B | $R_1$= $CH_3$, $R_2$=$R_3$=$R_4$=$R_5$=H | 5 |
| 3-(N-suberoyl argininyl)bufalin | B | $R_1$= $CH_3$, $R_2$=H, $R_3$=OAc | 2 |
| 3-(N-suberoyl argininyl) desacetylcinobufagin | B | $R_1$= $CH_3$, $R_2$=H, $R_3$=OH | 6 |
| 3-(N-succinyl argininyl)gamabufotalin | B | $R_1$=$CH_2$OH, $R_2$=H, $R_3$=OAc | 2 |
| 3-(N-succinyl argininyl) telocinobufagin | B | $R_1$= $CH_3$, $R_2$=OH, $R_3$=$R_4$=$R_5$=H | 6 |
| 3-(N-suberoyl argininyl) hellebrigenin | B | $R_1$=CHO, $R_2$=OH, $R_3$=$R_4$=$R_5$=H | 6 |
| 5-hydroxytryptamine | C | $R_1$=OH, $R_2$=$NH_2$ | - |
| bufotenine | C | $R_1$=OH, $R_2$=$N(CH_3)_2$ | - |
| bufotenine N-oxide | C | $R_1$=OH, $R_2$=$NOCH_3$ | - |
| bufobutanoic acid | C | $R_1$=OH, $R_2$= NH $CO(CH_2)_2COOH$ | - |
| bufotenidine | C | $R_1$=$O^-$, $R_2$= $N(CH_3)^{3+}$ | - |
| cholesterol | D | - | - |

**Table. S2:** Bufonis Venenum samples from different Origins

| Sample No. | Name | Date | Location |
|---|---|---|---|
| 1 | Shandong-1 | Oct, 2017 | Shandong |
| 2 | Shandong-2 | Apr, 2017 | Shandong |
| 3 | Shandong-3 | May, 2017 | Shandong |
| 4 | Linyi-1 | May, 2017 | Linyi, Shandong |
| 5 | Linyi-2 | Jan, 2017 | Linyi, Shandong |
| 6 | Nantong-1 | Oct, 2014 | Nantong, Jiangsu |
| 7 | Nantong-2 | June, 2015 | Nantong, Jiangsu |
| 8 | Nantong-3 | Aug, 2015 | Nantong, Jiangsu |
| 9 | Nantong-4 | Mar, 2016 | Nantong, Jiangsu |
| 10 | Nantong-5 | Feb, 2017 | Nantong, Jiangsu |
| 11 | Xuzhou-1 | Jan, 2017 | Sanyitang, Anhui |
| 12 | Xuzhou-2 | Jan, 2017 | Sanyitang, Anhui |
| 13 | Sanyiyang-1 | Jan, 2017 | Sanyitang, Anhui |
| 14 | Sanyiyang-2 | Jan, 2017 | Sanyitang, Anhui |
| 15 | Sanyiyang-3 | Jan, 2017 | Sanyitang, Anhui |
| 16 | Sanyiyang-4 | Jan, 2017 | Sanyitang, Anhui |
| 17 | Sanyiyang-5 | Jan, 2017 | Sanyitang, Anhui |
| 18 | Sanyiyang-6 | Jan, 2017 | Sanyitang, Anhui |
| 19 | Sanyiyang-7 | May, 2017 | Sanyitang, Anhui |
| 20 | Sanyiyang-8 | Jan, 2017 | Taiji, Chongqing |
| 21 | Taiji-1 | Jan, 2017 | Taiji, Chongqing |
| 22 | Taiji-2 | Jan, 2017 | Xuzhou, Jiangsu |
| 23 | Taiji-3 | Jan, 2017 | Xuzhou, Jiangsu |
| 24 | Taiji-4 | May, 2017 | Xuzhou, Jiangsu |
| 25 | Leiyunshang-1 | Aug, 2018 | Suzhou, Jiangsu |
| 26 | Leiyunshang-2 | Aug, 2018 | Suzhou, Jiangsu |
| 27 | Leiyunshang-3 | Aug, 2018 | Suzhou, Jiangsu |
| 28 | Leiyunshang-4 | Aug, 2018 | Suzhou, Jiangsu |
| 29 | Leiyunshang-5 | Aug, 2018 | Suzhou, Jiangsu |

**Table. S3*.** Standardized value of different cell inhibition rates

| | Hela | Tca-8113 | SK-OV-3 | mean | $x_i$* |
|---|---|---|---|---|---|
| resibufogenin | 16.37 | 11.02 | 10.48 | 12.63 | 0.2998 |
| cinobufagin | 43.11 | 60.39 | 40.15 | 47.88 | 1.1368 |
| bufalin | 63.38 | 62.07 | 48.47 | 57.97 | 1.3764 |
| bufotalin | 65.38 | 56.79 | 36.89 | 53.02 | 1.2588 |
| gamabufotalin | 42.15 | 28.91 | 32.01 | 34.36 | 0.8157 |
| arenobufagin | 61.19 | 56.82 | 36.43 | 51.48 | 1.2222 |
| desacety-bufotalin | 45.93 | 51.60 | 32.85 | 43.46 | 1.0318 |
| telocinobufagin | 38.82 | 54.44 | 30.60 | 41.29 | 0.9802 |
| cinobufotalin | 44.55 | 39.95 | 26.51 | 37.00 | 0.8785 |

Table. S3*: Quoted from the experimental data of Wang Jian, a student guided by Professor Ma Hongyue of the research group. $x_i$*: The standardized value of the inhibition rates of nine bufadienolides on different cells.

**Table S4.** Standardized value of correlation coefficient for PLS analysis.

| | VIP value | $y_i$* |
|---|---|---|
| resibufogenin | 2.45 | 1.023 |
| cinobufagin | 2.63 | 1.098 |
| bufalin | 2.08 | 0.868 |
| bufotalin | 2.97 | 1.240 |
| gamabufotalin | 2.35 | 0.981 |
| arenobufagin | 2.33 | 0.972 |
| desacety-bufotalin | 2.63 | 1.098 |
| telocinobufagin | 2.41 | 1.006 |
| cinobufotalin | 1.71 | 0.714 |

$y_i$*: The standardized value of the VIP value of the PLS analysis