

Supplementary Materials

Azasteroid Alkylators as Dual Inhibitors of AKT and ERK Signaling for the Treatment of Ovarian Carcinoma

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A

| | CONTROL | | ENGA-L06E (1h) | | ENGA-L06E (3h) | | ENGA-L08E (1h) | | ENGA-L08E (3h) | | |
|---------------|-----------|---------|----------------|--------|----------------|--------|----------------|--------|----------------|--------|------|
| | intensity | ratio | intensity | ratio | intensity | ratio | intensity | ratio | intensity | ratio | |
| OVCAR-3 cells | pAKT | 563180 | 1.21 | 289472 | 0.52 | 152939 | 0.22 | 274443 | 0.40 | 199215 | 0.34 |
| | pERK1,2 | 860724 | 1.85 | 414942 | 0.74 | 708919 | 1.02 | 345929 | 0.51 | 164940 | 0.28 |
| | GAPDH | 464712 | - | 561721 | - | 691668 | - | 683957 | - | 593050 | - |
| SKOV-3 cells | pAKT | 617312 | 0.996 | 215992 | 0.40 | 117254 | 0.20 | 118946 | 0.22 | 106241 | 0.20 |
| | pERK1,2 | 1104413 | 1.78 | 530139 | 0.99 | 222301 | 0.39 | 286115 | 0.54 | 257360 | 0.49 |
| | GAPDH | 619567 | - | 536577 | - | 576819 | - | 532376 | - | 522807 | - |

Data from western blots representing respective densitometry readings expressed as intensity values and ratios ([pAKT] or [pERK1+pERK2] / [GAPDH] band intensity).

B

| | | CONTROL | | ENGA-L06E | | ENGA-L08E | |
|---------------|---------|----------------|-------|----------------|-------|----------------|-------|
| OVCAR-3 cells | | Mean intensity | ratio | Mean intensity | ratio | Mean intensity | ratio |
| 30min | pAKT | 601325 | 1.23 | 430001 | 0.92 | 426975 | 0.93 |
| | pERK1,2 | 834569 | 1.71 | 655377 | 1.40 | 460820 | 1.01 |
| | GAPDH | 487948 | - | 467392 | - | 456755 | - |
| 1h | pAKT | 567151 | 1.14 | 359454 | 0.62 | 405926 | 0.60 |
| | pERK1,2 | 819566 | 1.65 | 556573 | 0.96 | 635950 | 0.94 |
| | GAPDH | 495672 | - | 579764 | - | 676543 | - |
| 3h | pAKT | 539850 | 1.27 | 427141 | 0.61 | 315402 | 0.52 |
| | pERK1,2 | 845927 | 1.99 | 777256 | 1.11 | 503430 | 0.83 |
| | GAPDH | 425564 | - | 700231 | - | 606542 | - |
| 6h | pAKT | 587921 | 1.26 | 228657 | 0.54 | 197866 | 0.47 |
| | pERK1,2 | 863557 | 1.85 | 225372 | 0.54 | 317632 | 0.76 |
| | GAPDH | 467213 | - | 420078 | - | 418763 | - |
| 12h | pAKT | 554331 | 1.18 | 187663 | 0.50 | 185206 | 0.48 |
| | pERK1,2 | 854432 | 1.82 | 184005 | 0.49 | 293415 | 0.76 |
| | GAPDH | 469805 | - | 378658 | - | 387541 | - |
| 24h | pAKT | 570034 | 1.29 | 209094 | 0.59 | 194218 | 0.54 |
| | pERK1,2 | 846679 | 1.92 | 207700 | 0.58 | 304429 | 0.84 |
| | GAPDH | 442047 | - | 354679 | - | 360356 | - |
| SKOV-3 cells | | Mean intensity | ratio | Mean intensity | ratio | Mean intensity | ratio |
| 15min | pAKT | 595562 | 0.98 | 523773 | 0.94 | 546274 | 1.04 |
| | pERK1,2 | 1029674 | 1.70 | 1064750 | 1.91 | 992317 | 1.89 |
| | GAPDH | 605638 | - | 556732 | - | 525870 | - |
| 30min | pAKT | 595562 | 0.98 | 435387 | 0.78 | 349409 | 0.66 |
| | pERK1,2 | 1029674 | 1.70 | 969159 | 1.74 | 847492 | 1.61 |
| | GAPDH | 605638 | - | 556732 | - | 525870 | - |
| 1h | pAKT | 557009 | 1.02 | 400360 | 0.73 | 371701 | 0.63 |
| | pERK1,2 | 969023 | 1.78 | 740686 | 1.35 | 636100 | 1.08 |
| | GAPDH | 545033 | - | 548965 | - | 587762 | - |

| | | | | | | | |
|-----|---------|---------|------|--------|------|--------|------|
| 3h | pAKT | 569703 | 0.99 | 376873 | 0.65 | 287955 | 0.52 |
| | pERK1,2 | 996401 | 1.74 | 392597 | 0.68 | 487004 | 0.89 |
| | GAPDH | 573459 | - | 578540 | - | 548799 | - |
| 12h | pAKT | 584509 | 0.94 | 302956 | 0.58 | 259479 | 0.50 |
| | pERK1,2 | 1002453 | 1.61 | 341709 | 0.65 | 442756 | 0.85 |
| | GAPDH | 623461 | - | 524054 | - | 518875 | - |

Data from pAKT and pERK1,2 time-depended inhibition curves representing densitometry readings expressed as mean intensity values and ratios ([pAKT] or [pERK1+pERK2] / [GAPDH] band intensity).

Figure 1. Densitometry readings / intensity ratios of expression and quantification data of Figure 3.

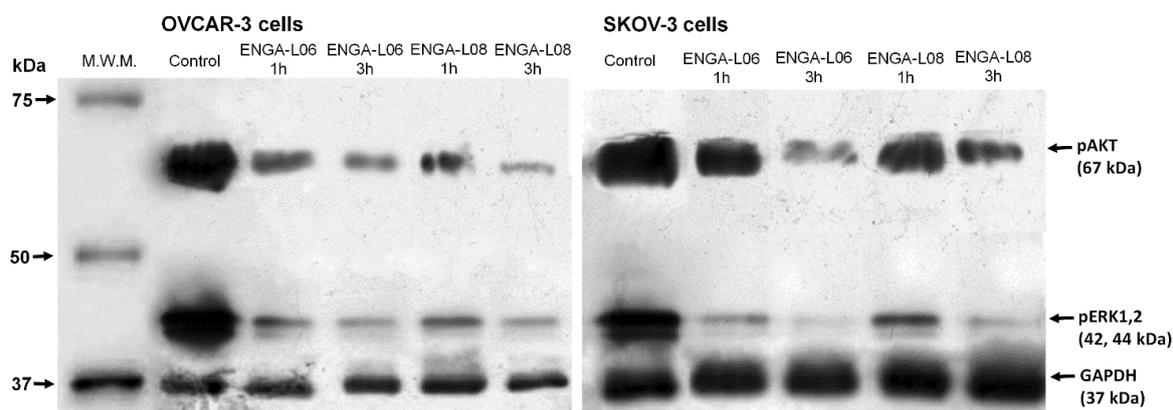


Figure 2. Whole western blots, with molecular weight markers (M.W.M.), presenting the expression levels of pERK 1,2 and pAKT in SK-OV-3 and OVCAR-3 ovarian cancer cell lines after treatment with hybrid aza-steroidal alkylators ENGA-L06E and ENGA-L08E at a concentration of 25 μ M in 1 and 3h intervals.