Supplementary data

Figure Legends

Figure S1:

A: Chemical structure of archazolid A

B: Alkalization of lysosomes by archazolid treatment

T24, MDA-MB-231 and 4T1 cells were treated with different archazolid concentrations (5 and 10nM) for 2h (T24 cells) and 4h (MDA-MB-231 and 4T1). Thereafter cells were stained for 30min at 37°C with a lysotracker dye (Molecular Probes) to monitor lysosomal pH using confocal microscopy.

Figure S2:

A: T24 cells stimulated with archazolid for 24h show no sign of apoptosis

Adherent and floating T24 cells were treated with archazolid for 24h (10nM). Apoptotic cell death was investigated as described in materials and methods. Bars represent the percentage of apoptotic cells as mean ± SEM of three independent experiments conducted in triplicates.

B: Concanamycin impairs colony formation in T24 cells

T24 cells were pretreated with concanamycin (1, 5 and 10nM, 24h) or left untreated (Co) and subsequently cultured in a soft agar layer for nine days. Anchorage independent growth was analyzed by counting stained colonies (MTT). Bars represent the percentage of colony formation compared to control. ***P< 0.001, n=3.

Figure S3: Adherent T24 cells stimulated with archazolid for 48h are highly apoptotic

Adherent and floating T24 cells were treated with archazolid for 48h (1, 2, 10 and 20nM). Apoptotic cell death was investigated as described in materials and methods. Bars represent the specific apoptotic rate compared to control as mean ± SEM of three independent experiments done in triplicates. ***P<0.05.

Figure S4: 4T1 cells show no cell death induction after 24h archazolid treatment

Adherent 4T1 cells were treated with archazolid for 24h (10nM). Apoptotic cell death was investigated as described in “materials and methods”. Bars represent the percentage of apoptotic cells as mean ± SEM of three independent experiments done in triplicates. 4T1 cells showed no sight of cell death after 24h treatment

Figure S5: BIM levels after archazolid treatment in MDA-MB-231 and MCF7 cells

Floating MDA-MB-231 and MCF7 cells were incubated with archazolid (10nM) for the indicated time. Western blot analysis of BIM expression was performed either in the mitochondrial fraction or the whole cell lysate. COX IV and β-Tubulin were used as loading control. All Western blots show a representative blot out of two independent experiments.

MCF7 cells were purchased from the DSMZ (Braunschweig, Germany) in 2009.

Figure S6: Quantification of BIM rescue by proteasome inhibitors

A combination of archazolid (10nM, 24h, T24 cells) with proteasome inhibitor MG-132 (left) or bortezomib (BOR, 48h) (right) was used to investigate changes in BIM protein level by Western blot. Three independent experiments were quantified using Image J software.

Figure S7:

A: ROS induction in floating 4T1 cells.

Archazolid induces generation of ROS in 4T1 cells after 16h of treatment. ROS generation was measured as indicated in “materials and methods”. Bars represent the mean ± SEM of three independent experiments conducted in triplicates. ***P<0.001.

B: ROS levels of archazolid treated, floating cells reached ROS levels of untreated adherent cells

ROS induction by archazolid in floating T24 cells was investigated after 24h treatment compared to floating and adherent control cells. Bars represent the relative level of ROS compared to control cells (floating). ***P<0.001, n=3

Figure S8: Phosphorylation of Akt and ERK is increased early after detachment but decreases again after 24h

A: T24 cells were kept in suspension for 6h and 24h and were compared to adherent cells. Phosphorylation of Akt was analyzed by Western blot. Actin served as loading control. One representative blot out of three independent experiments is shown.

B: T24 cells were kept in suspension for 3, 5h and 24h in comparison to adherent cells. Phosphorylation of ERK and c-Src was analyzed by Western blot. Actin served as loading control. One representative blot out of three independent experiments is shown.

C: Quantification of Akt induction

Akt kinase activation by phosphorylation was investigated after 48h of archazolid treatment by Western blot. Three independent experiments were quantified using Image J software.

Figure S9: The inhibitors LY-294002 (Akt), PD 98059 (ERK) or Saracatinib (c-Scr) induce apoptosis in floating cancer cells

Floating T24 cells were treated with Akt-, ERK-, and Src-inhibitors for 48h and apoptotic cell death was analyzed. Bars represent the percantage of apoptotic cells of the whole population in mean ± SEM of three independent experiments conducted in triplicates. ***P<0.001.

Figure S10: Anoikis resistance and archazolid action on anoikis resistant cancer cells

Left: Altered signaling pathways to prevent anoikis: Cancer cells can inhibit anoikis induction by activating integrin and integrin downstream targets like FAK, Src, Akt and ERK. Thereby preventing BIM transcription and translocation to the mitochondria. Caspase-8 inhibition is also crucial to prevent anoikis.

Right: Archazolid induces anoikis: Active integrin and the downstream target FAK are inhibited by archazolid treatment. Additionally caspase-8 is activated and the caspase-8 inhibitor FLIP is reduced. Archazolid triggers an early induction and translocation of BIM to the mitochondria. Countermechanisms to archazolid treatment are the induction of Akt and

the prominent removal of BIM. Prolonged archazolid treatment leads to anoikis in anoikis resistant cancer cells.