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# Supplementary Materials

*Article*

## The artemisinin-derived autofluorescent compound BG95 exerts anticytomegaloviral activity based on a mitochondrial targeting mechanism

**Markus Wild[1], Friedrich Hahn[1*], Benedikt Grau[2], Lars Herrmann[2], Aischa Niesar[1], Martin Schütz[1], Mélanie M. Lorion[3], Lutz Ackermann[3], Svetlana B. Tsogoeva[2], Manfred Marschall[1,*]**

[1] Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), Schlossgarten 4, 91054 Erlangen, Germany; markus.wild@uk-erlangen.de, friedrich.hahn@uk-erlangen.de, aischa.niesar@uk-erlangen.de, manfred.marschall@fau.de

[2] Institute of Organic Chemistry I, FAU, Nikolaus-Fiebiger-Straße 10, 91058 Erlangen Germany; benedikt.grau@fau.de, lars.herrmann@fau.de, svetlana.tsogoeva@fau.de

[3] Institute for Organic and Biomolecular Chemistry, Georg-August-Universität Göttingen, Tammannstraße 2, 37077 Göttingen, Germany; melanie.lorion@chemie.uni-goettingen.de, lutz.ackermann@chemie.uni-goettingen.de

* Correspondence: manfred.marschall@fau.de (M.M.), friedrich.hahn@uk-erlangen.de (F.H.); Tel.: ++49 9131 8526089 (M.M.), ++49 9131 8536480

**Table S1.** Characteristics of mitochondria under treatment with BG95/ART compounds in HFFs and U373 cells.[#]

| Cell type | Occurrance of BG95 bodies | Colocalization of BG95 bodies with prohibitin | HCMV-induced dispersed mitochondria | BG95-induced punctate mitochondria | BG95-related compounds also induce punctate mitochondria |
|---|---|---|---|---|---|
| HFF | + | + | + | + | + |
| U373 | + | + | nd* | nd* | nd* |

[#] For evaluation of characteristics see Figures 2-5, 7 and S3-S5.
* nd, not determined

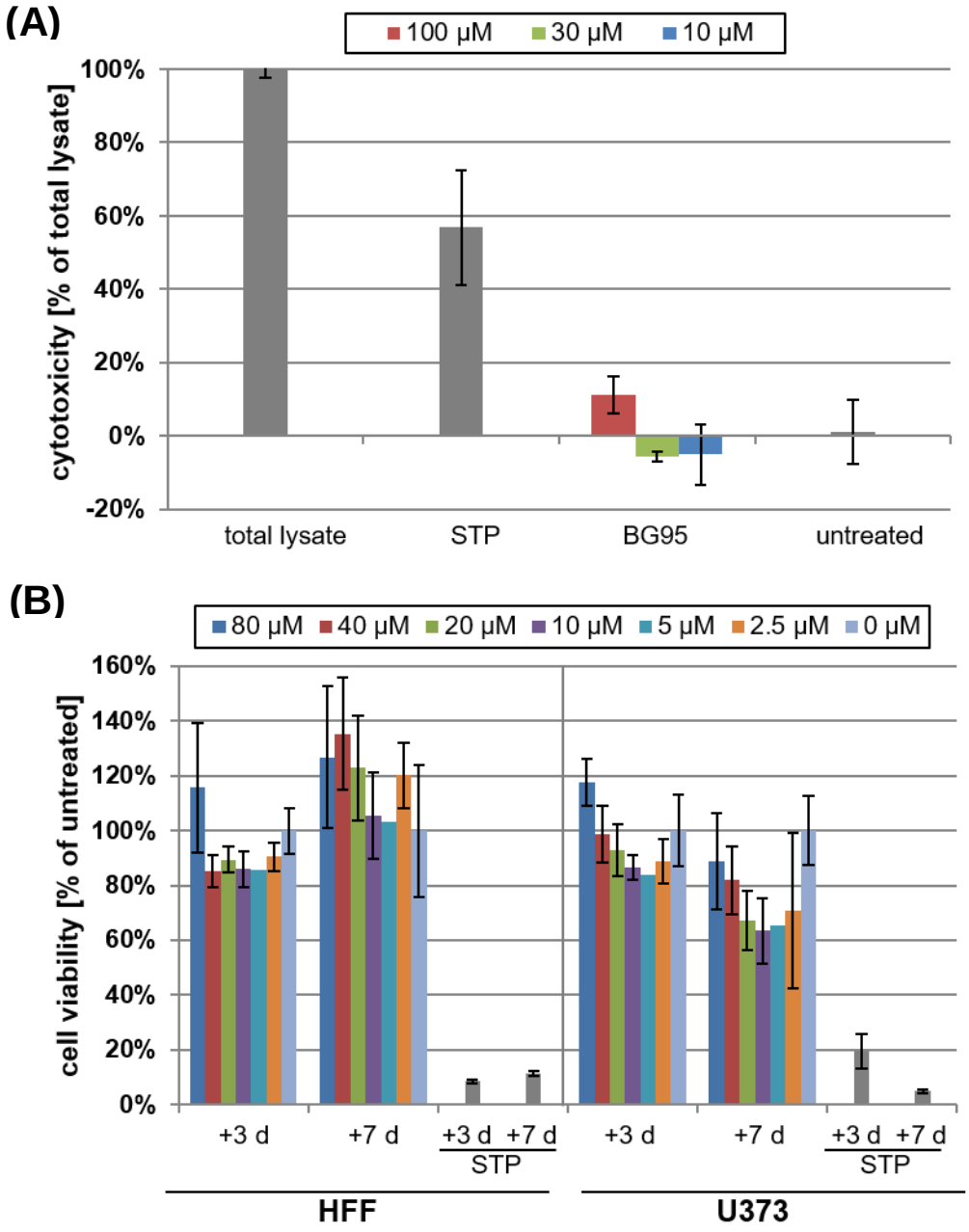


**Figure S1.** BG95 shows no cytotoxic activity after incubation periods of 1, 3 and 7 d. **(A)** Lactate dehydrogenase release assay was performed under standard conditions using cell culture media supernatants of HFFs treated with the indicated concentrations of BG95 for 1 d. As a positive control, the cytotoxicity-inducing kinase inhibitor staurosporine (STP, 0.1 µM) was used. **(B)** Neutral red cytotoxicity assay was performed on HFF or U373 cell layers treated with the indicated concentrations of BG95 for 3 d and 7 d, using 1 µM of STP as a positive control.

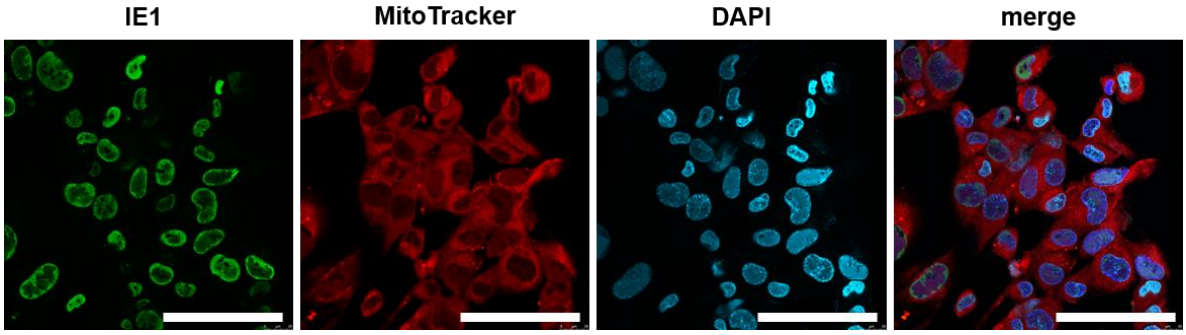


**Figure S2.** Productive infection of HFFs with HCMV strain AD169 was visualized by the detection of IE1 expression. HFFs were infected at an MOI of 2, fixed 4 d p.i. and IE1 expression detected by immunofluorescence staining using MAb-IE1 antibody (p63-27). Scale bars represent 100 μm.

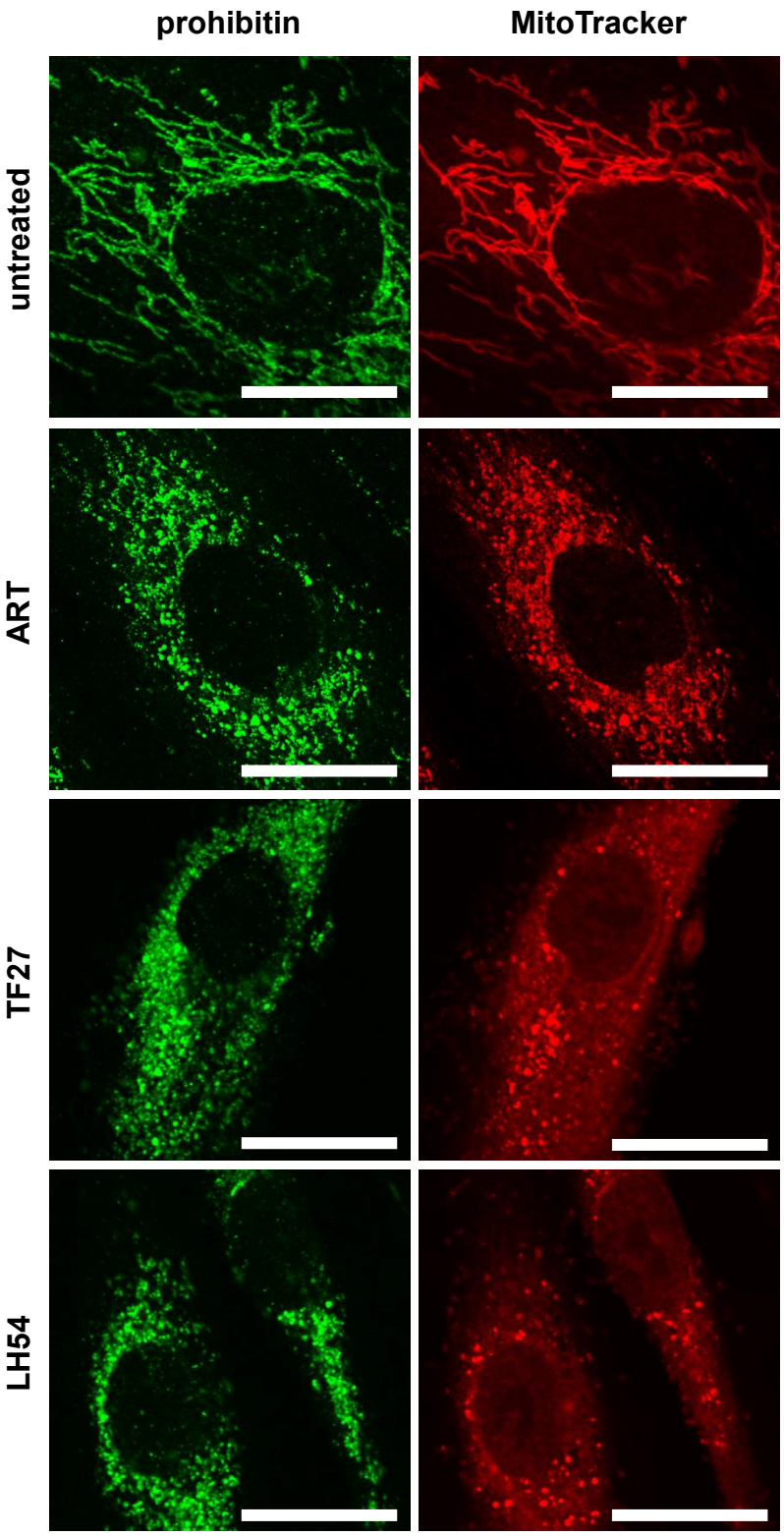


**Figure S3.** Artesunate and its derivatives TF27 and LH54 elicit changes to mitochondrial architecture of HFFs. Loss of the characteristic filamentous structure and fragmentation of mitochondria into a punctate phenotype was observed upon treatment with 10 μM of ART, TF27 and LH54, similar to those induced by BG95 treatment. Scale bars represent 25 μm.

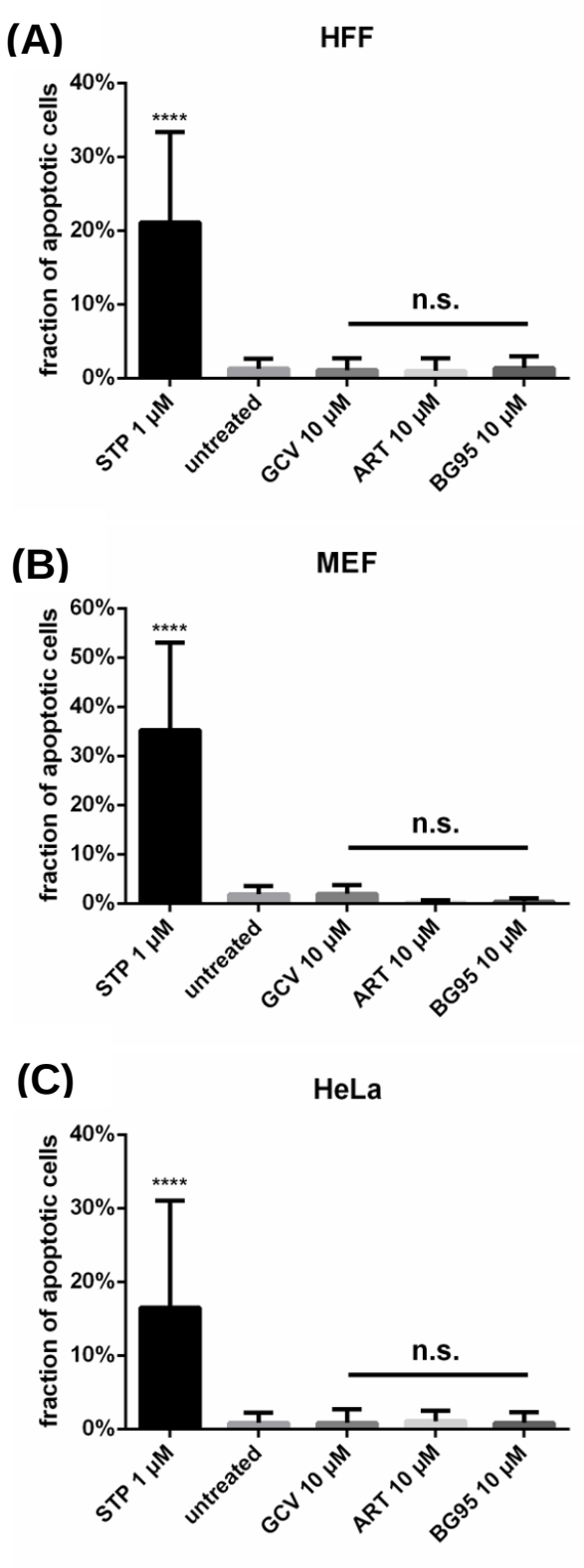


**Figure S4.** BG95 does not induce apoptosis in primary human or murine fibroblasts, i.e., HFFs **(A)** or MEFs **(B)**, and HeLa cells **(C)**. Cells were treated with the indicated concentrations of compounds or remained untreated for 3 d, after which they were subjected to NucView staining, fixation and DAPI staining. The fraction of NucView-positive cells was counted in at least 20 pictures per compound. Data is given as the mean of the fractions of individual pictures + SD. STP was used as a positive control (1 µM, added 4 h before fixation). Statistical analysis was performed using ordinary One-way ANOVA followed by post-hoc Tukey's test compared to untreated. ****, $p < 0.0001$; n.s., not significant.

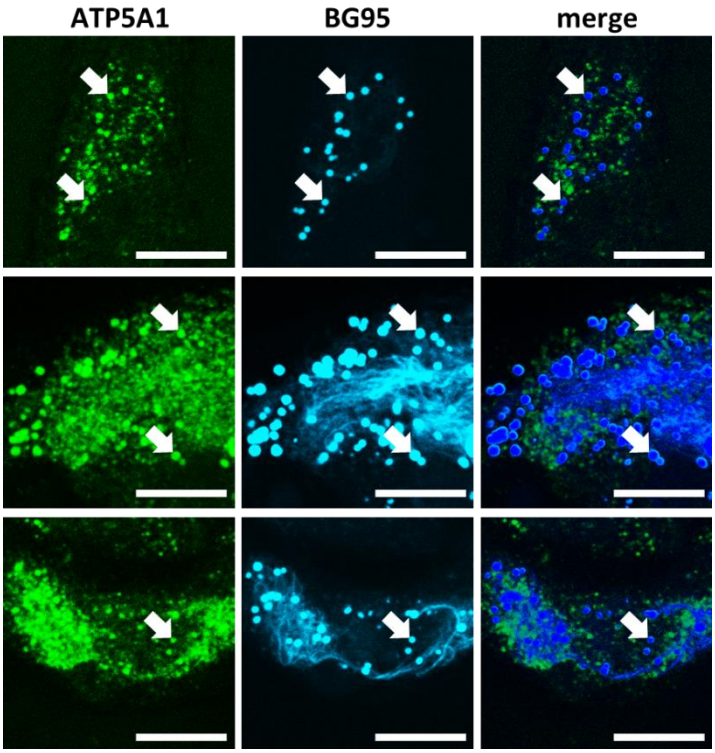


**Figure S5.** Autofluorescent BG95 accumulates in characteristic intramitochondrial bodies as indicated by the ATP5A1 marker protein. HFFs were infected with HCMV AD169 (MOI 2), treated with 10 μM of BG95, fixed at 3 d p.i. and immunostained using an ATP5A1-specific antibody (7H10BD4F9, Invitrogen). At 3 d p.i., BG95 bodies colocalized with mitochondrial marker ATP5A1 (similar to prohibitin, see Figs. 2, 3 and 5). White arrows indicate BG95 bodies, referring to the corresponding positions in other channels. Note the appearance of additional, so far uncharacterized thread-like autofluorescent structures particularly in the lower two panels. Scale bars represent 20 μm.