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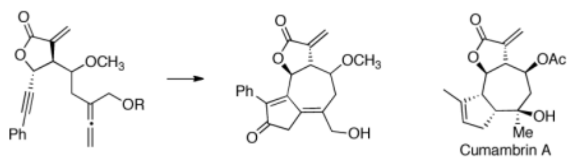
A Redox Economical Synthesis of Bioactive 6,12-Guaianolides

Bo Wen[†], **Joseph K. Hexum**[‡], **John C. Widen**[‡], **Daniel A. Harki**[‡], and **Kay M. Brummond**[†]
Daniel A. Harki: daharki@umn.edu; Kay M. Brummond: kbrummon@pitt.edu
[†]Department of Chemistry, Univ. of Pittsburgh, Pittsburgh, PA 15260
[‡]Department of Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55414

Abstract



Syntheses of two 6,12-guaianolide analogs are reported within. The scope of the tandem allylboration/lactonization chemistry is expanded to provide a functionalized alleneyne-containing α-methylene butyrolactone that undergoes a Rh(I)-catalyzed cyclocarbonylation reaction to afford a 5-7-5 ring system. The resulting cycloadducts bear a structural resemblance to other NF-κB inhibitors such as cumambrin A and indeed were shown to inhibit NF-κB signaling and cancer cell growth.

Guaianolides are the most abundant group of sesquiterpene lactones (SLs), possessing a privileged natural product status and a wide range of biological activities.[1] Yet there is only one guaianolide, arglabin, available as a marketed drug, constituting one of only twenty-four natural products approved for therapeutic use between 1974 and 2006. [2, 3] Reasons for the slow realization of their therapeutic potential include poor bioavailability due to high plasma protein interactions, poor toxicological profiles, and hydrophobicity. [4] Moreover, the biological activity of these compounds is attributed to covalent bonding to the α,β-unsaturated carbonyl groups, the same functionality responsible for their toxicity.[5] Despite potential toxicities, three of the top-ten drugs in the US, and one third of all enzyme targets for which there is an FDA approved inhibitor, operate by a covalent mechanism of action.[6] These proven biomedical applications, combined with the finding that irreversible binding may be an important factor against drug resistance, have led to a reinvestment of the pharmaceutical community in covalent drugs.[6,7]

Natural products, such as guaianolides can serve as excellent leads for drug development, but molecular complexity can pose formidable synthetic challenges.[8] To date, most synthetic approaches towards 6,12-guaianolides can be characterized as target-oriented synthesis (TOS) strategies that have not been explored for analog preparation of these highly oxygenated skeletons;[9] the synthesis of thapsigargin (**2**) being one exception (Figure 1).[10] Oxidation level [O] constitutes one measure of molecular complexity which can be directly correlated with synthetic accessibility when performing a TOS.[11] For example, the synthetic

Correspondence to: Daniel A. Harki, daharki@umn.edu; Kay M. Brummond, kbrummon@pitt.edu.

Supporting Information Available Detailed procedures and data for all compounds in Schemes 2–4, biochemical assays and Figures S1–S3 are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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steps required to prepare arglabin (**8**) and chinensiolide (**7**) where [O] = 4, were fewer than twenty. In contrast, more than forty steps were required to complete the synthesis of thapsigargin (**2**).[10] Given the highly oxidized nature of 6,12-guaianolides, a synthetic approach employing the principles of redox economy would greatly alleviate the synthetic challenges associated with the class of compounds.[11]

Described within is an eleven-step synthesis of two guaianolide analogs with oxidation levels equivalent to thapsigargin and eupatochinilide VI; concise syntheses that were realized by limiting the number of redox adjustments in the synthetic sequence. We have previously demonstrated the advantages of early-stage incorporation of an α-methylene butyrolactone on the Rh(I)-catalyzed allenic Pauson-Khand reaction (APKR).[12] This study expands on the scope of the APKR by incorporating additional functionality into the alleneyne precursor **10**. Furthermore, bioactivity studies provides support for the preparation of non-naturally occuring guaianolide analogs such as **11** (Scheme 1).[13]

Synthesis of alleneyne **10** was envisioned using the allylboration/lactonization chemistry developed by Hall and previously used by us to access less functionalized alleneyne precursors. Because there is only one report with functionality at a propargylic position, a model system was first examined.[14] Compounds **12a-d** were prepared and converted to the corresponding carbomethoxy allylboronates **13a-d** by addition of DIBAL and subsequent trapping of the intermediate aluminum species with $ClCH_2BPin$ (Scheme 2). CuI was not required for the 1,4-addition reaction of hydride to the ynoate, possibly because the ether adjacent to the alkyne directs the addition. Moreover, *Z:E* ratios of allylboronates **13a-d** were dependent upon the protecting group. For example, the reaction of **12a-b**, with silyl protecting groups afforded **13a-b** in *Z:E* ratios of 2–3:1. Whereas, reaction of methyl- and MOM-protected ethers, **12c** and **12d**, afforded the allylboronates **13c** and **13d** with *Z:E* ratios of 9:1 and 4:1, respectively. The stereochemical determining step is the addition of the electrophile to one face over the other of the intermediate allenoate **14**. We propose that the *Z:E* ratios correlate with the degree of chelation of the respective ether groups with the aluminum species of the allenoate, where more chelation directs electrophilic addition to the α-face.[15]

Next, the lactonization step was examined on these model systems (Scheme 3). Unfortunately, the *E:Z* isomers of allylboronate **13** were not readily separated by column chromatography so they were taken on to the lactonization step as a mixture. Reaction of allylboronates **13a** or **13b**, with either a TBS or TBDPS protecting group with boron trifluoride etherate, triflic acid or scandium triflate gave only decomposition. However, reaction of allylboronate **13c** with either triflic acid or scandium triflate gave ~75% yield of **15c** in a 3–4:1 *trans:cis* lactone ratio. For the MOM-protected ether **13d**, purely thermal conditions gave the best results, whereby heating **13d** and phenylpropiolaldehyde to 90 °C for 48 h gave an 82% yield of **15d** as a *trans:cis* ratio of 2.7:1; acidic conditions led to decomposition of **13d**. Next, the feasibility of allylation/lactonization chemistry was tested on a more functionalized substrate.

To this end, allenyl ester **16** is obtained in 82% yield from the mono-protected butynediol using a Johnson-Claisen rearrangement (Scheme 4). Ester **16** is reacted with methoxymethylamine hydrochloride and isopropyl magnesium chloride to afford the corresponding Weinreb amide in 84% yield, which is taken on to alkynone **17** by reaction with ethynyl magnesium bromide. Reduction of the carbonyl of ynone **17** is accomplished with lithium aluminum hydride in 98% yield. The propargylic alcohol is not purified but taken directly on to the corresponding methyl ether in 90% yield. Deprotonation of the terminus of the alkyne with n-butyllithium followed by addition of chloromethylester gives the alkynoate **18** in 84% yield. Reaction of alkynoate **18** with Dibal-H, CuI, MeLi, and

$ClCH_2BPin$ afforded allylboronate **19** in 78% yield with a *Z:E* ratio of 1.2:1. Performing this reaction in the absence of MeLi and CuI gave allylboronate **19** in 80% yield with a *Z:E* ratio of 2.2:1 with more byproduct contamination. The *Z:E* isomers were not separated, but taken on as a mixture to the allylboration/lactonization step. Reaction of **19** with phenylpropiolaldehyde using the acidic conditions described above all gave decomposition of the allylboronate. Purely thermal conditions in toluene afforded starting material at 50 °C and decomposition at 90 °C. Interestingly, heating allylboronate **19** with 3-phenylpropiolaldehyde in chloroform for 7 days afforded some of the desired lactones **20a-b**, but the bulk of the material consisted of intermediate hydroxy esters.[16] This complex mixture was reacted with PTSA to afford lactone *trans*-**20a** as 2:1 mixture of diastereomers in 40% yield. Uncyclized material was recovered after chromatography and reacted with NaH to afford lactone *cis*-**20b** as a single diastereomer in 14% yield. The *cis*- and *trans*-lactones were taken on independently to the Rh(I)-catalyzed cyclocarbonylation reaction. Reaction of **20a** with rhodium biscarbonyl chloride dimer in toluene at 90 °C afforded the cyclocarbonylation product **21a** as a mixture of diastereomers. The *tert*-butyldiphenylsilyl (TBDPS) group of **21a** was removed using triethylamine hydrogen fluoride to give **22a** in 64% yield for the two steps. Reaction of the *cis*-lactone **20b** to the same sequence afforded **22b** in 37% yield (two steps).

Natural products bearing α-methylene butyrolactones are well-established bioactive molecules. Inhibition of the NF-κB signaling pathway, a hingepoint for the activation of the cellular inflammatory response, has been demonstrated by molecules of this class.[17] In addition, it has been shown that a natural product analog, dimethylamino-parthenolide (DMAPT; LC-1), has the ability to simultaneously knockdown NF-κB levels and activate the p53 pathway, thus promoting the apoptosis of cancer cells.[18] Inspired by these previous studies, we evaluated **22a-b** for inhibition of induced NF-κB activity in cell cultures. A549 cells bearing a stably transfected NF-κB reporter construct were treated with each compound. Activation of NF-κB signaling yields an increase in reporter luminescence that is diminished in the presence of NF-κB inhibitors.[19] Results from our study were benchmarked against parthenolide (**PTL**), a known NF-κB inhibitor bearing an α-methylene butyrolactone. Trans-**22a** and cis-**22b** were equipotent inhibitors in this assay, diminishing induced NF-κB activity to non-induced levels at 20 μM. Both analogs resulted in substantial decreases in NF-κB activity, with 57% (**22a**) and 59% (**22b**) residual activity measured at 10 μM. **PTL** was found to be slightly more potent, reducing NF-κB levels to 53% residual activity at 10 μM.

Inhibition of NF-κB signaling is an emerging strategy for developing novel anticancer agents. [20] Additionally, many α-methylene butyrolactone-containing natural products have documented antiproliferative activities.[13] We evaluated **22a-b** for growth inhibitory activity against a panel of cancerous and non-cancerous cell lines. Both compounds were benchmarked against **PTL** and clinically used drugs gemcitabine and doxorubicin (Figures S1 and S2). Antiproliferative data for **PTL** has been previously reported for HL-60, HeLa, U-87 MG, and Vero and our data is in close agreement to previous reports. [21] In general, **22a** and **22b** were similarly active when compared to each other, and slightly less active than **PTL**. Notable exceptions to this trend include HeLa breast carcinoma and HL-60 leukemia cells, in which **22a** was approximately 2-fold more active than **PTL (**Table 1). Conversely, *cis*-**22b** was significantly more active than *trans*-**22a** in U-87 MG brain tumor cells (IC_{50} = 9.8 μM versus 27.1 μM) and has similar activity to **PTL** (IC_{50} = 8.8 μM). Interestingly, **22b** (IC_{50} = 25.4 μM) was significantly more active than both **22a** (IC_{50} = 80.9 μM) and **PTL** (IC_{50} = 57.6 μM) against the well-known NCI/ADR-RES cell line, which is a model of drug-resistant ovarian cancer due to overexpression of p-glycoprotein (P-gp) efflux pump. [22] NCI/ADR-RES is resistant to doxorubixin (adriamycin) and

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gemcitabine (IC_{50}'s > 500 μM, Figure S2). These results suggest that molecules with covalent mechanisms of activity, such as the guaianolide analogs **22a-b**, may be valuable scaffolds for targeting drug-resistant cells. Both compounds were screened against the non-cancerous cell line Vero and moderate toxicity was observed for all α-methylene butyrolactone analogs.

In conclusion, the scope of the APKR has been extended to the preparation of highly oxygenated guaianolide analogs, **22a-b**. Bioactivity data supports the potential of this class of compounds as regulators of NF-κB and cell proliferation, and further validates the medicinal relevancy of this region of chemical space. Our ability to modify the structure of these compounds *de novo* enables the optimization of analog solubility and pharmacokinetic properties for advanced biological applications. Furthermore, our strategy provides ready access to uniquely functionalized 6,12-guaianolide analogs with activities on par with the most studied member of the SLs, parthenolide. Studies are underway to establish structure activity relationships and the mechanism by which compounds **22a-b** inhibit NF-κB, in addition to benchmarking their thiol reactivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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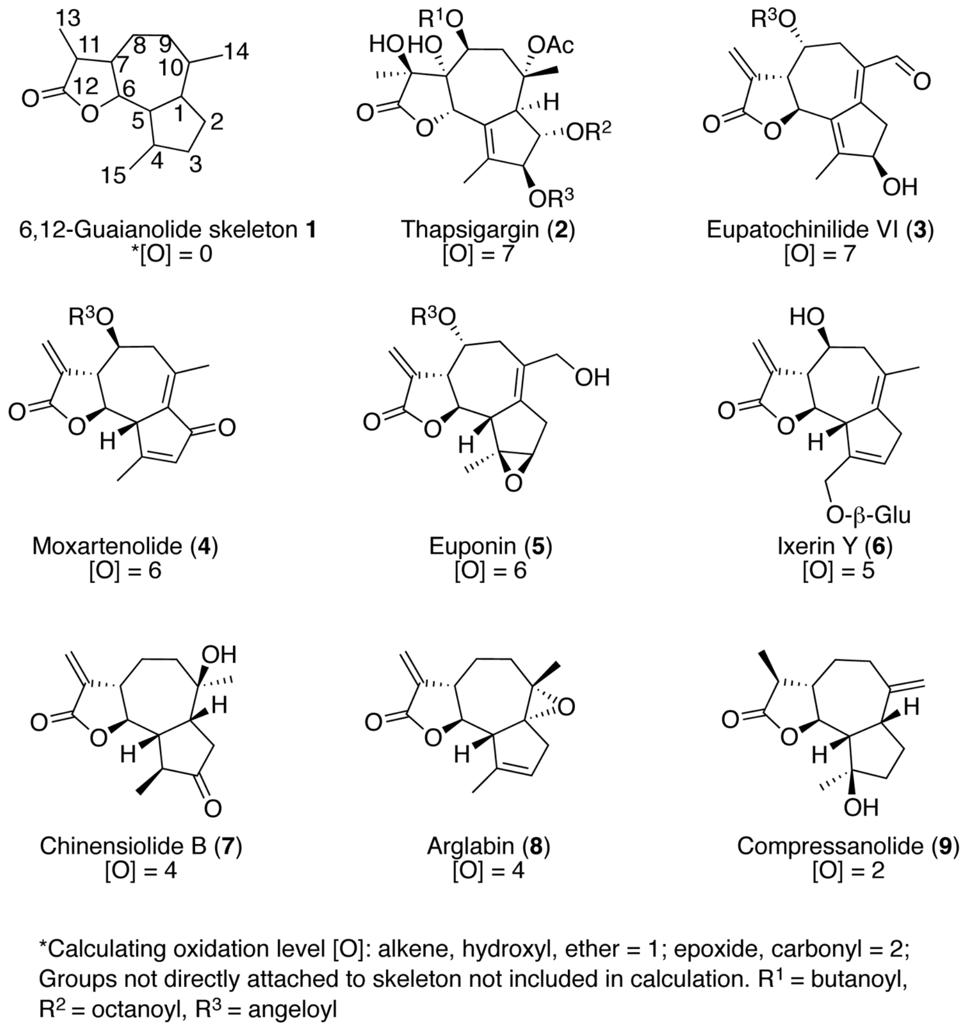

Figure 1.
Examples of highly oxidized 6,12-guaianolides

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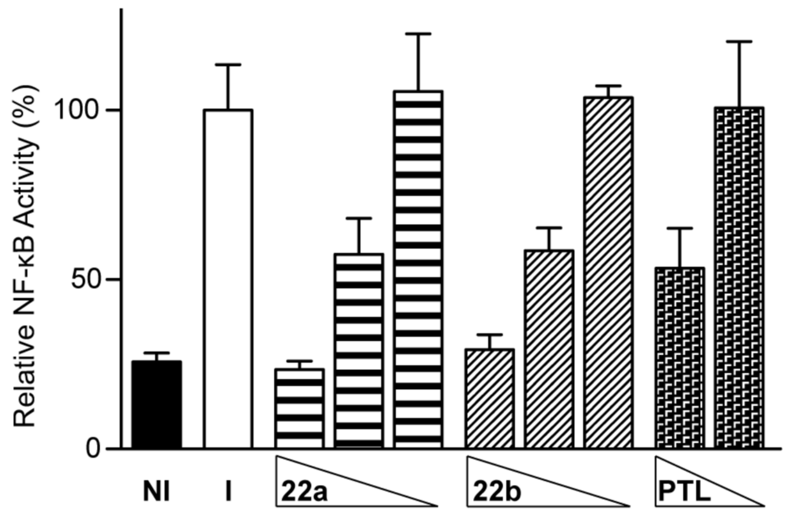

Figure 2.

NF-κB luciferase reporter assay in A549 cells. Compounds **22a-b** were dosed at 20, 10, and 1 μM and **PTL** was dosed at 10 and 1 μM. Cells were induced with TNF-α (15 ng/mL) 30 min after molecule treatment, except NI control. Shown is the mean of triplicate data and error bars represent propagated standard deviation. NI = non-induced, I = induced.

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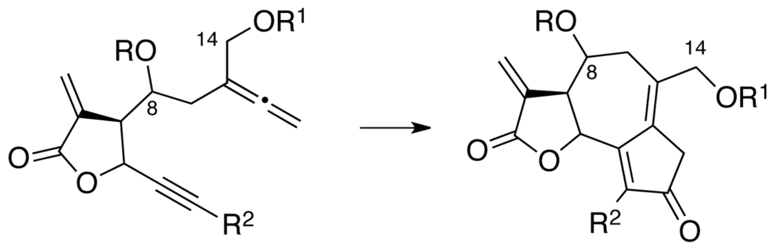

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Scheme 1.
An APKR approach to highly oxidized guaianolides

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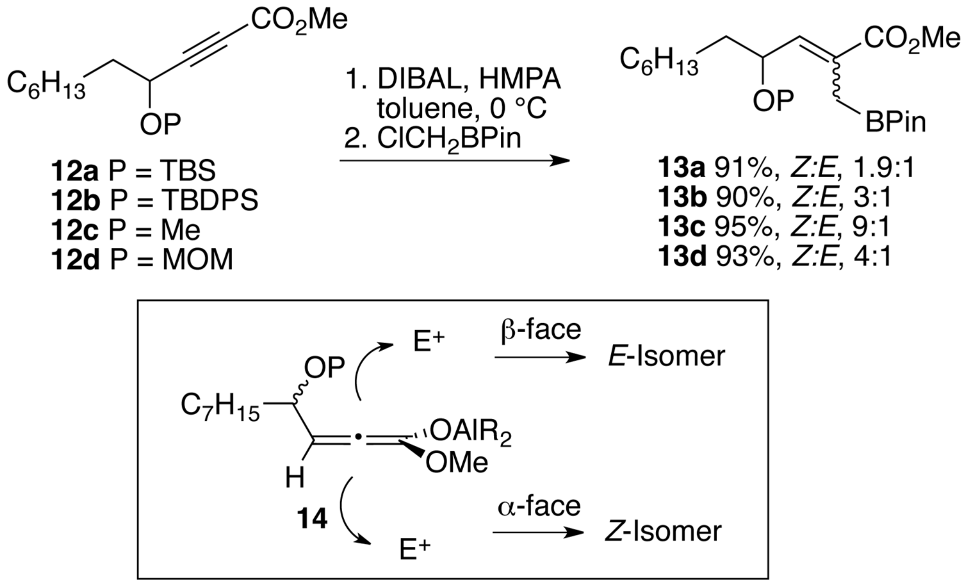

Scheme 2.

Generation of the allylboronates, *Z:E* ratios

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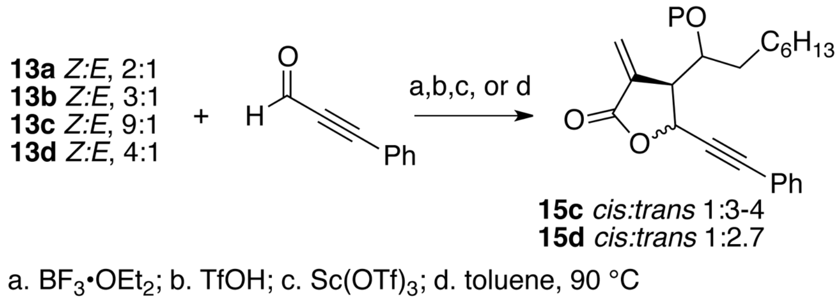

Scheme 3.
A model system for the lactonization protocol

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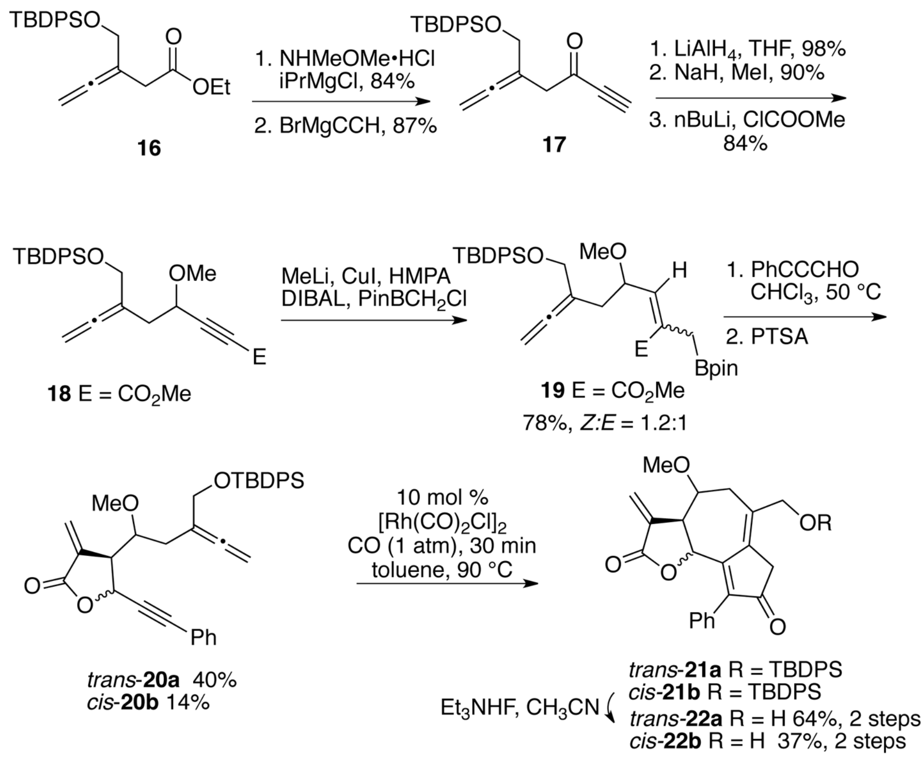

Scheme 4.

Synthesis of 6,12-guaianolide analogs **22a-b**

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Table 1

Antiproliferative activities of **22a-b** and parthenolide (**PTL**)[a]

compound	DU-145	HeLa	HL-60	U-87 MG	NCI/ADR-RES	Vero
22a	29.1 ± 4.7	20.3 ± 6.0	5.5 ± 0.4	27.1 ± 4.8	80.9 ± 24.0	32.2 ± 7.0
22b	21.6 ± 1.9	39.7 ± 16.4	7.8 ± 2.3	9.8 ± 1.4	25.4 ± 1.0	30.1 ± 5.5
PTL	8.9 ± 4.6	45.1 ± 3.7	9.3 ± 3.8	8.8 ± 2.1	57.6 ± 8.9	22.4 ± 1.5

[a]Compounds were dosed to cells and incubated for 48 h. Viability was measured by Alamar Blue staining. Mean IC_{50} values ± S.D. (μM) are shown.