SIG1459: A novel anti-acne isoprenylcysteine compound

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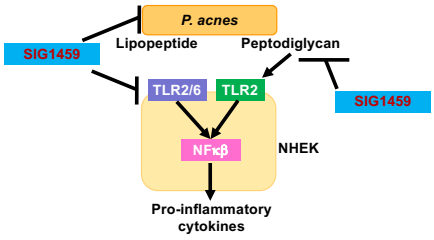
Abstract

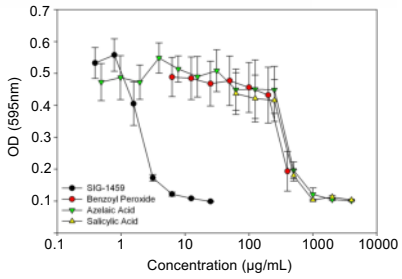
Propionibacterium acnes is a major contributing factor to acne vulgaris, a common disorder that is estimated to affect 9.4% of the global population. *P. acnes* colonize and proliferate within the pilosebaceous follicles causing the induction of local inflammatory response. This is mediated through the interaction of *P. acnes* with epidermal keratinocytes leading to activation of toll-like receptors and later resulting in the production and secretion of pro-inflammatory mediators. Isoprenylcysteine (IPC) small molecules represent a novel class of topically applied non-steroidal anti-inflammatories that can be used in consumer products and more potent derivatives for drug development to treat skin diseases. Here we report IPC derivative, SIG1459, downregulates these inflammatory signaling pathways and directly decreases *P. acnes* viability. Keratinocytes exposed to *P. acnes*, peptidoglycan and FSL-1 released pro-inflammatory cytokines and were inhibited by SIG1459 with IC_{50} values in the nanomolar range. In an *in vitro* growth inhibition assay of cultured *P. acnes*, SIG1459 outperformed anti-acne agents, benzoyl peroxide (BPO) and salicylic acid, exhibiting a strong MIC of 5 µM, MBC of 10 µM, and eradicating *P. acnes* biofilm formation (MBEC) at 21 µM. Lastly, 1% SIG1459 formulation in an 8-week single blind, vehicle controlled study was shown to significantly outperform 3% BPO using an investigator global assessment acne scale. SIG1459 (n= 35) resulted in a ~2-grade reduction after 8 weeks, while BPO (n=15) resulted in a 1.5-grade reduction. Moreover, SIG1459 was well tolerated with no adverse reactions (stinging, burning, dryness, staining), which as expected were reported by subjects in BPO arm of the study. These data demonstrate that phytyl-cysteine derived IPCs, SIG1459 represent a novel chemical-class that provides a dual modulating benefit to acne by limiting bacterial proliferation and inhibiting inflammation.

Fig 1. IPCs target both *P. acnes* induced inflammation and growth



Propionibacterium acnes (P. acnes) is a major contributing factor to the inflammatory component of acne. The interaction of bacterial cell-wall components including peptidoglycan (PGN) and lipopeptides with keratinocytes (NHEK) leads to an innate immune response via activation of toll-like receptors (TLR2, TLR2/6) resulting in the production and secretion of pro-inflammatory mediators. Phytyl-cysteine compound SIG1459 derived from our IPC library platform inhibits both *P. acnes*-induced cytokine production and growth.

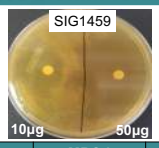
Fig 2. SIG1459 demonstrates strong antimicrobial activity versus *P. acnes*



Compound	MIC µg/mL (µM)
SIG1459	3 (5)
Benzoyl Peroxide	200 (826)
Salicylic Acid	1000 (7240)
Azelaic Acid	1000 (5313)
Clindamycin	0.06 (0.1)

P. acnes (ATCC® 6919™) cultures (10^6 CFU/mL) were incubated with compounds in 5% DMSO under anaerobic conditions at 37ºC for 72 hours. After incubation, OD_{595nm} of each sample was measured to determine bacterial growth. MIC was defined as the lowest concentration of an agent that achieved ≥90% eradication of visible growth. Data represent average results from 3 independent experiments.

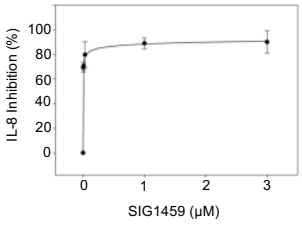
Fig 3. SIG1459 eradicates *P. acnes* biofilm formation



Compound	MBC [a]	MBEC [b]
	µg/mL (µM)	
SIG1459	6 (10)	12 (21)
Benzoyl Peroxide	200 (826)	367 (1515)
Salicylic Acid	>2000 (>14480)	>8000 (>57920)
Azelaic Acid	>2000 (>10626)	8000 (42503)
Clindamycin	1 (2)	0.6 (1)

[a] *P. acnes* (ATCC® 6919™) was cultured as described for MIC determination. [b]MBC range was defined as the minimal concentration of compound that caused complete inhibition of colony forming units (CFUs) of *P. acnes* as compared to the control after 72 hours incubation with each compound. [b] Bacteria biofilms were established by seeding *P. acnes* cultures in 96-well plates and incubating for 24 hours without agitation. Later, biofilms were incubated with test materials for 24 hours. Remaining biofilms were washed and stained with crystal violet. Staining solution was removed, wells rinsed with water and dye was extracted with 1% w/v SDS. The absorbance was measured at 595 nm in a microplate reader. MBEC was defined as the minimum concentration necessary to achieve ≥80% eradication of attached biofilm compared to vehicle-only control. [b] Results from disk diffusion susceptibility testing (Kirby-Bauer Method) after 72 hours incubation.

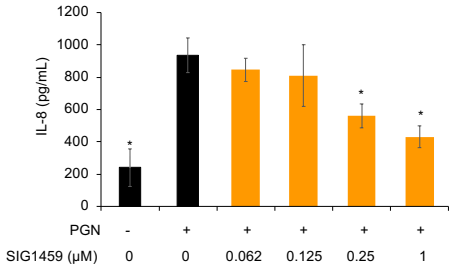
Fig 4. SIG1459 inhibits *P. acnes*-induced cytokine release



Compound	IL-8 IC_{50} (nM)
SIG1459	3
Clobetasol	33

Normal Human Epidermal Keratinocytes (NHEKs) were pre-treated with test compounds for 2 hours and later cultured for 24 hours with *P. acnes* live bacteria (10^3 CFU/NHEK) and co-treated with test compounds. Interleukin-8 (IL-8) levels were measured by ELISA method. Data represent average results from 3 independent experiments. IC_{50} values were determined by non-linear regression analysis using the four-parameter logistic equation.

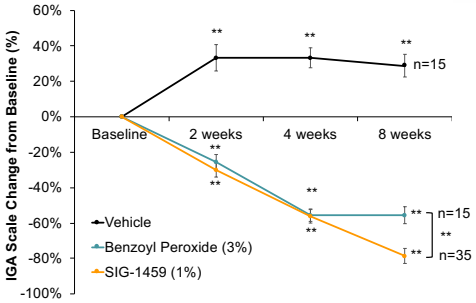
Fig 5. SIG1459 attenuates TLR2 and TLR2/6-induced IL-8 production



Compound	IL-8 inhibition IC_{50} (µM)	
	PGN	FSL1
SIG1459	0.25	0.5
Benzoyl Peroxide	>1	>10
Salicylic Acid	>1	>25

Normal Human Epidermal Keratinocytes were pre-treated with test compounds for 2 hours and later cultured for 24 hours with TLR2 agonist (PGN, 10 µg/mL) or TLR2/6 agonist (FSL1 , 0.1 µg/mL) and co-treated with test compounds. Interleukin-8 (IL-8) levels were measured by ELISA method. Data represent average results from 3 independent experiments. IC_{50} values were determined from curve fitting using 4-parameter logistic equation. * p value ≤ 0.05 by Student *t* test compared to PGN- or FSL1-only treated cells.

Fig 6. SIG1459 (1%) outperforms BPO (3%) in an 8-week acne clinical tolerance study



A multi-site use single-blinded study was conducted in healthy male and female subjects, aged ≥18 yo with evaluator assessed mild to moderate acne, to evaluate the potential efficacy of test skincare product by utilizing subjective questionnaires, visual evaluations and digital photography (≥15 per group). Subjects used the assigned product at home for 8 weeks. Subjects returned post baseline at week 2, 4 and 8. At each visit subjects underwent expert clinical grading and test site photography. At Visit 4, subjects also completed a Self-Perception Questionnaire (SPQ). *Values are given as mean ± S.E. * p value ≤ 0.05; ** p value ≤ 0.01 by Student *t* test between group differences from IGA scale values from baseline.

Fig 7. SIG1459 1% cream is well tolerated and effective in acne prone skin subjects

Facial Cream (1% SIG1459) was tested in a randomized single-blind vehicle-controlled study (Active, n=35; Vehicle, n=15) to demonstrate the safety and tolerability in subjects with mild to moderate facial acne. The severity of acne signs and symptoms on the faces of ≥18 yo subjects were clinically assessed by IGA scale during an 8-week Study period. In addition, UV light mode was utilized to observe porphyrins fluorescence (orange-red dots). Reduction of porphyrins is an indirect measure of *P. acnes* killing. Several subjects using the SIG1459 facial cream demonstrated marked visual improvement in the signs and symptoms of acne as well as reduction in porphyrins during and after weeks 2-8 of application.

Summary/Conclusions

- **Isoprenylcysteine (IPC) compound SIG1459 dose-dependently inhibits keratinocyte IL-8 secretion in response to *P. acnes*, TLR2 and TLR2/6 heterodimer specific ligands, suggesting the potential for inhibition of the initial neutrophil infiltration on *P. acnes* exposure by modulating keratinocyte TLR2 signaling**
- **SIG1459 has antimicrobial activity against *P. acnes*: inhibiting its growth, demonstrating bactericidal activity and blocking biofilm formation better than current cosmetic anti-acne actives**
- **SIG1459 is well tolerated clinically in human subjects with acne prone skin and significantly outperforms Benzoyl Peroxide on the acne IGA clinical scale at week 2 and week 8. Moreover, a reduction in porphyrins on the face, is observed suggesting a reduction in *P. acnes* counts *in vivo*, supporting *in vitro* findings**
- **IPC compounds represent a novel class of anti-acne molecules derived from our IPC technology platform. SIG1459 and its derivatives provide safe, dual modulating benefits to combat acne**