SIG-1459 and SIG-1460: Novel anti-acne phytyl-cysteine compounds

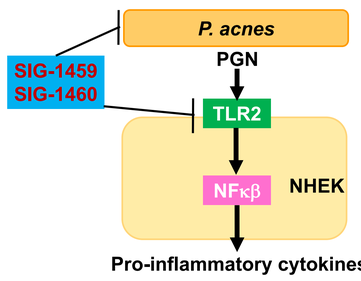
José R. Fernández[1], Karl Rouzard[1], Corey Webb[1], Michael Voronkov[1], Jason Healy[1], Masanori Tamura[1], Kristen L. Huber[1], Jeffry B. Stock[1,2] Maxwell Stock[1], Joel S. Gordon[1], Eduardo Pérez[1]

[1]Signum Dermalogix, 133 Wall Street, Princeton, NJ; [2]Princeton University, Department of Molecular Biology, Princeton, NJ

Abstract

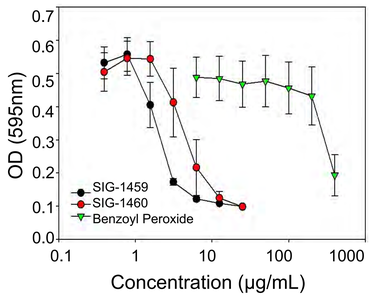
Propionibacterium acnes (P. acnes) is a major contributing factor to acne vulgaris, a common disorder among postpubescent teens that is estimated to affect 9.4% of the global population. Comedones, the primary acne lesions, are the result of abnormal follicular keratinization related to excessive sebum secretion. *P. acnes* colonize and proliferate within the pilosebaceous follicles causing the induction of a local inflammatory response. This is mediated through the interaction of *P. acnes* with epidermal keratinocytes leading to activation of toll-like receptor (TLR2) and later resulting in the production and secretion of pro-inflammatory mediators. For the first time, we report phytyl-cysteine based compounds derived from our isoprenylcysteine (IPC) technology platform, SIG-1459 and SIG-1460, downregulate these inflammatory signaling pathways and directly decrease *P. acnes* viability. Cultured human keratinocytes were exposed to *P. acnes* and peptidoglycan (PGN) to induce pro-inflammatory cytokine production. In these cell based models, SIG-1459 and SIG-1460 inhibited IL-8 production versus both TLR2 inducers. In an *in vitro* growth inhibition assay of cultured *P. acnes*, SIG-1459 and SIG-1460 both outperform commonly applied anti-acne agents, benzoyl peroxide and salicylic acid, exhibiting a minimal inhibitory concentration (MIC) of 3-4 µg/mL. These data demonstrate phytyl-cysteine compounds, SIG-1459 and SIG-1460 represent a novel chemical-class that provides a dual modulating anti-acne benefit 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 lipopolysaccharides (LPS) with keratinocytes (NHEK) leads to an innate immune response via activation of toll-like receptors (TLR2, TLR4) resulting in the production and secretion of pro-inflammatory mediators. Phytyl-cysteine compounds (SIG-1459, SIG-1460) derived from our IPC library platform inhibits both *P. acnes*-induced cytokine production and growth.

Fig 2. SIG-1459 and SIG-1460 have antibacterial activity versus *P. acnes*



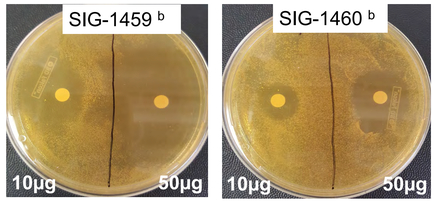
Compound	MIC [a]	MBC [b]
	µg/mL (µM)	
SIG-1459	3 (5)	6 (10)
SIG-1460	4 (6)	25 (40)
Benzoyl Peroxide	200 (826)	200 (826)
Salicylic Acid	1000 (7240)	>2000 (>14480)
Azelaic Acid	1000 (5313)	>2000 (>10626)
Clindamycin	0.06 (0.1)	1 (2)

[a] *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. [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. Data represent average results from 3 independent experiments.

Fig 3. SIG-1459 and SIG-1460 inhibit *P. acnes* biofilm formation

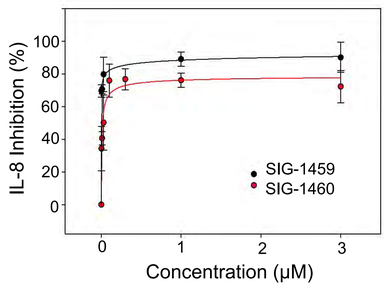
Compound	MBEC [a]
	µg/mL (µM)
SIG-1459	12 (21)
SIG-1460	7 (11)
Benzoyl Peroxide	367 (1515)
Salicylic Acid	>8000 (>57920)
Azelaic Acid	8000 (42503)
Clindamycin	0.6 (1)

Disk Diffusion for MBC Determination (Fig. 2)



[a] *P. acnes* (ATCC® 6919™) was cultured as described for MIC determination. 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.

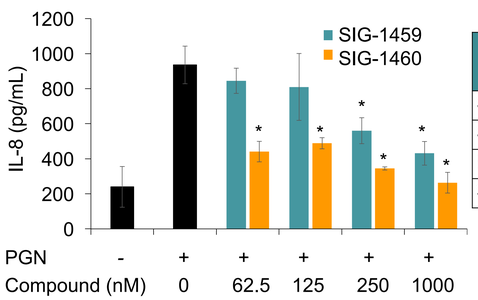
Fig 4. SIG-1459 and SIG-1460 inhibit *P. acnes*-induced cytokine release



Compound	IL-8
	IC_{50} (nM)
SIG-1459	3
SIG-1460	68
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 and SIG1460 attenuate TLR2 induced IL-8 production

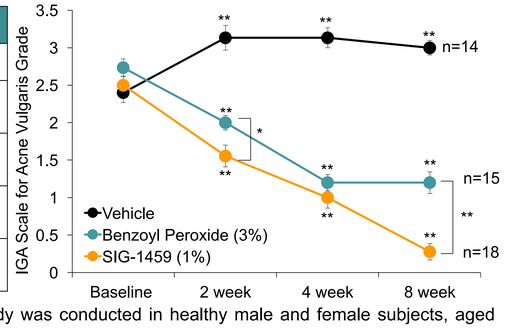


Compound	IL-8
	IC_{50} (nM)
SIG-1459	250
SIG-1460	50
Benzoyl Peroxide	>1000
Salicylic Acid	>1000

Normal Human Epidermal Keratinocytes were pre-treated with test compounds for 2 hours and later cultured for 24 hours with TLR-2 agonist (PGN, 10 µg/mL) and co-treated with Phytyl-cysteine 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-only treated cells.

Fig 6. SIG-1459 (1%) outperforms BPO (3%) in an 8 week clinical tolerance study

IGA Scale	Description
0	no inflammatory or non-inflammatory lesions
1	few non-inflammatory lesions with no more than one small inflammatory lesion
2	some non-inflammatory lesions with no more than a few inflammatory lesions
3	Moderate severity and many non-inflammatory lesions and may have some inflammatory lesions
4	Severe and many non-inflammatory and inflammatory lesions, but no more than a few nodular lesions



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. SIG-1459 1% cream is well tolerated and effective in acne prone skin subjects

Facial Cream (1% SIG-1459) was tested in a randomized single-blind vehicle-controlled study (Active, n=18; Vehicle, n=14) 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). Several subjects using the SIG-1459 facial cream demonstrated marked visual improvement in the signs & symptoms of acne as well as reduction in porphyrins during and after weeks 2-8 of application.

Summary/Conclusions

- **Phytyl-cysteine compounds (SIG-1459, SIG-1460) dose-dependently inhibit keratinocyte IL-8 secretion in response to both *P. acnes* and TLR2 specific ligand, suggesting the potential for inhibition of the initial neutrophil infiltration on *P. acnes* exposure by modulating keratinocyte TLR2 signaling**
- **SIG-1459 and SIG-1460 have antimicrobial activity against *P. acnes*: inhibiting its growth, demonstrating bactericidal activity and blocking biofilm formation better than current anti-acne actives**
- **SIG-1459 is well tolerated clinically in human subjects with acne prone skin and significantly outperforms BPO 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**
- **Phytyl-cysteine compounds represent a novel class of anti-acne molecules derived from our IPC technology platform. SIG-1459 and SIG-1460 provide safe, dual modulating benefits to combat acne**