P01-53

Anti-inflammatory, Anti-Aging and Anti-Bacterial Properties of SIG1273: A Skin Protecting Cosmetic Functional Ingredient

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Abstract

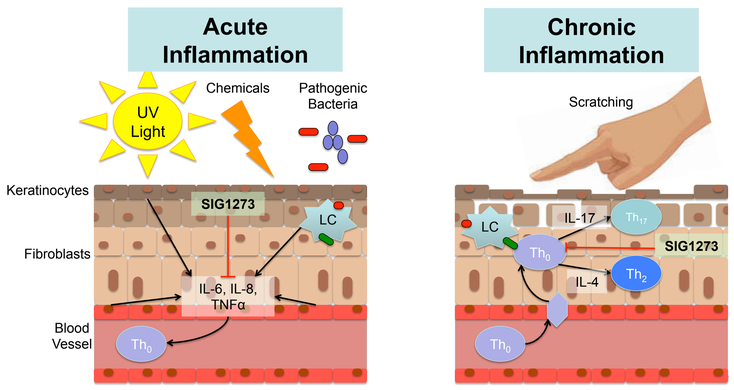
Background: The skin is the first line of defense against exposure to microbial, physical, environmental and chemical insults. In mobilizing a protective response, several different cell types located in our skin release and respond to pro-inflammatory cytokines ensuring skin homeostasis and health. However, chronic activation of this response, eventually causes damage resulting in premature aging. Tetramethylhexadecenyl Succinoyl Cysteine (SIG1273), an isoprenylcysteine small molecule down modulates these inflammatory signaling pathways in various cell types and possesses anti-bacterial properties.

Methods: NHEKs, were exposed to chemical irritant phorbol 12-myrisate 13-acetate (TPA) or Ultraviolet-B light (UVB) to induce pro-inflammatory cytokine (IL-6, IL-8 and TNF-α) production. T-cell receptor (TCR) activation of PBMCs and nickel treatments of HDMECs were performed resulting in IL-4, IL-6, IL-8 and IL-17 production. *Streptococcus pyogenes* were cultured to determine minimal inhibitory concentration (MIC) values.

Results: *In vitro* studies demonstrate SIG1273 blocks TPA and UVB-induced cytokine production in cultured keratinocytes. Similarly, SIG1273 inhibits overproduction of IL-4 and IL-17 in T-cell Receptor (TCR)-activated PBMCs as well as nickel induction of IL-6 and IL-8 in HDMECs. Lastly, SIG1273 demonstrated antimicrobial properties, inhibiting cell growth of *S. pyogenes and P. acnes*.

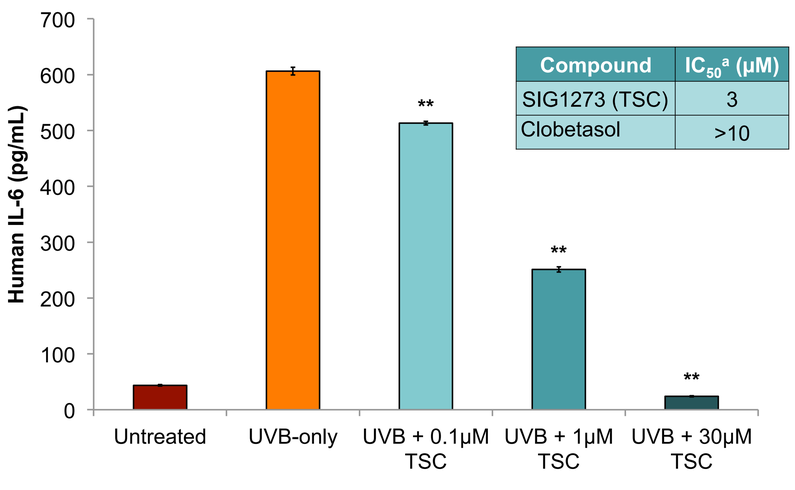
Conclusions: SIG1273 represents a novel cosmetic functional ingredient that provides a broad spectrum of benefits for the skin.

Fig 1. SIG1273 targets elicited inflammatory signals



Skin inflammation could be triggered by environmental exposure of skin to ultraviolet (UV) light, harmful bacteria overgrowth or chemical/metal contact leading to chronic irritation. Acute inflammation is mediated by damaged keratinocytes and their release of pro-inflammatory cytokines to initiate the innate immune response. During chronic inflammation a sustained T-cell mediated long lasting response is maintained, thus a resulting in tissue damage. SIG1273 is a novel CFI which inhibits pro-inflammatory cytokine production from different skin-related cell types and inhibits pathogenic bacteria growth.

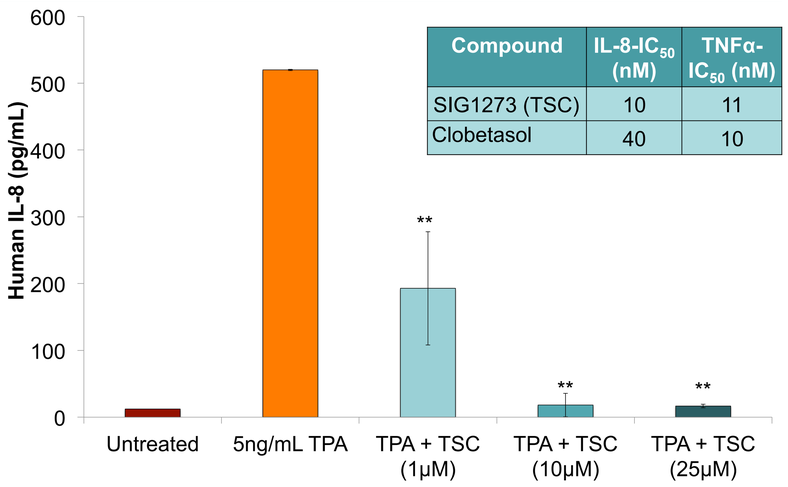
Fig 2. SIG1273 inhibit UVB-induced IL-6 secretion in human keratinocytes



Compound	IC_{50}[a] (μM)
SIG1273 (TSC)	3
Clobetasol	>10

Primary Human Epidermal Keratinocytes (NHEKs) were pre-treated with SIG1273 for 6 hr, then irradiated with 25 mJ/cm^2 broadband 305-12nm UVB (Daavlin; Bryan, OH) and later incubated for 24 hours. Subsequently, IL-6 production was measured by ELISA. All compounds were tested at the concentrations shown. No cell cytotoxicity of NHEKs was observed at concentrations shown. The IC_{50} is the concentration of compound producing half maximal inhibition. * p value < 0.05; ** p value < 0.01.

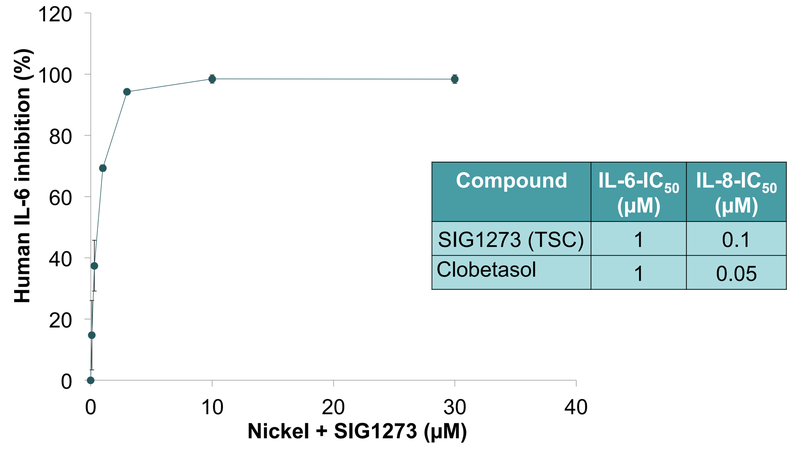
Fig 3. SIG1273 inhibit TPA-induced IL-8 and TNF-α secretion in NHEKs



Compound	IL-8-IC_{50} (nM)	TNFα-IC_{50} (nM)
SIG1273 (TSC)	10	11
Clobetasol	40	10

Primary Human Epidermal Keratinocytes (NHEKs) were pre-treated with SIG1273 for 2 hours, then co-treated with 5ng/mL TPA (Sigma-Aldrich) for 6 hours. Subsequently, IL-8 and TNF-α production was measured by ELISA. All compounds were tested at the concentrations shown. No cell cytotoxicity of NHEKs was observed at concentrations shown. The IC_{50} is the concentration of compound producing half maximal inhibition. * p value < 0.05; ** p value < 0.01.

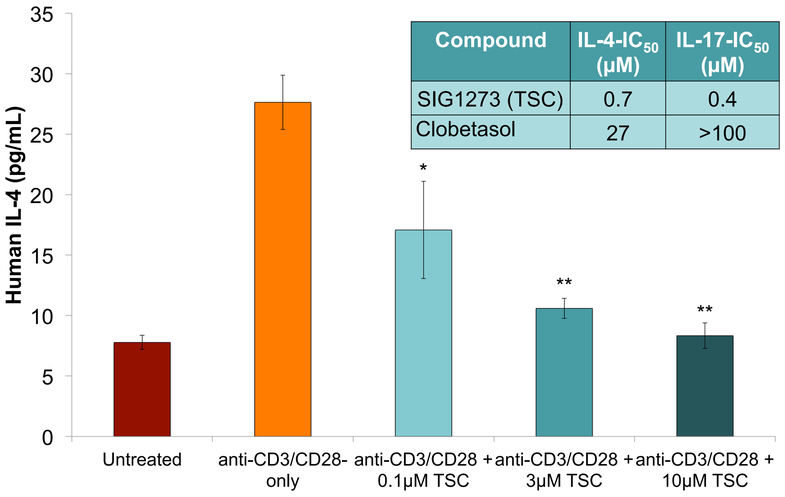
Fig 3. SIG1273 inhibit Nickel-induced IL-6 secretion in HDMECs



Compound	IL-6-IC_{50} (μM)	IL-8-IC_{50} (μM)
SIG1273 (TSC)	1	0.1
Clobetasol	1	0.05

Primary Human Dermal Microvascular Endothelial Cells (HDMECs) were pre-treated with SIG1273 for 2 hours, then co-treated with 1mM $NiSO_4$ (Sigma-Aldrich) for 6 hours. Subsequently, IL-6 production was measured by ELISA. All compounds were tested at the concentrations shown. No cell cytotoxicity of NHEKs was observed at concentrations shown. The IC_{50} is the concentration of compound producing half maximal inhibition. * p value < 0.05; ** p value < 0.01.

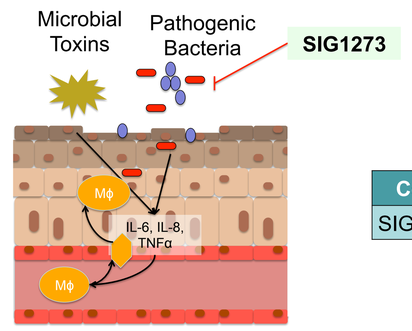
Fig 5. SIG1273 inhibit TCR-induced IL-4 and IL-17 in hPBMCs



Compound	IL-4-IC_{50} (μM)	IL-17-IC_{50} (μM)
SIG1273 (TSC)	0.7	0.4
Clobetasol	27	>100

Primary human Peripheral Blood Mononuclear Cells (hPBMCs) were pre-treated with SIG1273 for 2 hours, then co-treated with anti-CD3/CD28 dynabeads® (Life Technologies) for 24 hours. Subsequently, IL-4 and IL-17 production was measured by ELISA. All compounds were tested at the concentrations shown. No cell cytotoxicity of NHEKs was observed at concentrations shown. The IC_{50} is the concentration of compound producing half maximal inhibition. * p value < 0.05; ** p value < 0.01.

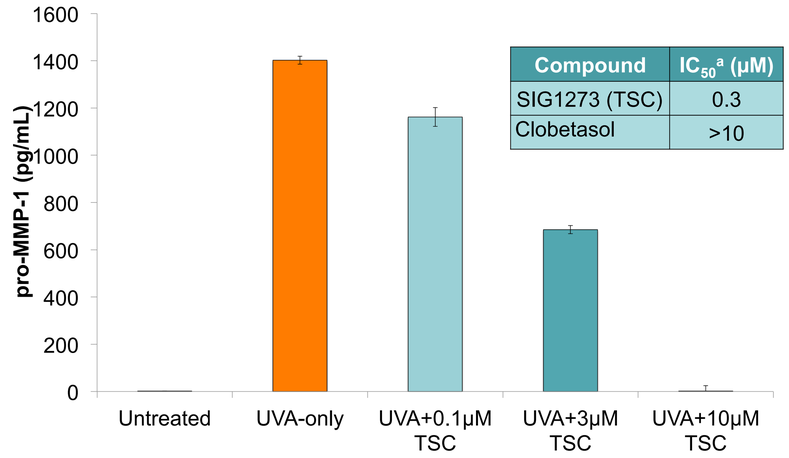
Fig 6. SIG1273 possess antimicrobial activity against skin pathogens



Compound	MIC (μg/mL)	
	P. acnes	*S. pyogenes*
SIG1273 (TSC)	2	2

S. pyogenes (ATCC 19615) was grown in tryptic soy agar with 5% BAP at 35-37ºC, in carbon dioxide. MIC testing for *S. pyogenes* was performed by the CRO ATS Labs (Eagan, MN). *P. acnes* (ATCC 6919) was grown under anaerobic conditions at 37ºC for 72 hours. TSC was diluted in DMSO to make 2 mg/ml stock solution. Aliquots of 10 µl of each TSC dilution (2-fold, e.g. 100, 50, 25, down to 0.8 µg/ml) were then added to two empty rows of eight wells on the microtiter plate. Aliquots of diluted cultured bacteria were added to 96-well plates and measure changes in Optical Densities at 600nm.

Fig 7. SIG1273 inhibit UVA-induced MMP-1 secretion in HDFs



Compound	IC_{50}[a] (μM)
SIG1273 (TSC)	0.3
Clobetasol	>10

Primary Human Dermal Fibroblasts (HDFs) were pre-treated with SIG1273 for 6 hr, then irradiated with 12.5 J/cm^2 UVA (350-12nm; Daavlin; Bryan, OH) and later incubated for 24 hours. Subsequently, pro-MMP-1 production was measured by ELISA. All compounds were tested at the concentrations shown. No cell cytotoxicity of HDFs was observed at concentrations shown. The IC_{50} is the concentration of compound producing half maximal inhibition. * p value < 0.05; ** p value < 0.01.

Summary/Conclusions

- **SIG1273 inhibits pro-inflammatory cytokine secretion in response to UVB, TPA, Nickel and at the T-cell receptor suggesting inhibition at different skin-related cell types by modulating both acute and chronic inflammation signaling.**
- **SIG1273 has antimicrobial action and potency against skin bacteria (*S. pyogenes and P. acnes*) similar to doxycycline.**
- **SIG1273 was shown to inhibits collagenase (MMP-1) secretion in response to UVA, suggesting ECM remodeling protection and anti-aging benefits.**
- **SIG1273 is a novel anti-inflammatory, anti-aging, anti-microbial cosmetic functional ingredient.**

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COI Disclosure

Jose R. Fernandez, PhD

In connection with the presentation, I disclose COI with the following company:

Full time Employee: Signum Dermalogix, Inc.