sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. *Proc Natl Acad Sci USA* 103:9903–7

Deavall DG, Martin EA, Horner JM, Roberts R (2012) Drug-induced oxidative stress and toxicity. *J Toxicol* 2012:1–13

Flaherty KT (2010) Narrative review: BRAF opens the door for therapeutic advances in melanoma. *Ann Intern Med* 153:587–91

Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of Atp-dependent transporters. *Nat Rev Cancer* 2:48–58

Grossman D, Altieri DC (2001) Drug resistance in melanoma: mechanisms, apoptosis, and new potential therapeutic targets. *Cancer Metastasis Rev* 20:3–11

Huang V, Hepper D, Anadkat M, Cornelius L (2012) Cutaneous toxic effects associated with vemurafenib and inhibition of the BRAF pathway. *Arch Dermatol* 148:628–33

Joseph EW, Pratilas CA, Poulikakos PI, Tadi M, Wang W, Taylor BS *et al.* (2010) The RAF inhibitor PLX4032 inhibits ERK signaling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc Natl Acad Sci USA* 107:14903–8

Mitsuishi Y, Taguchi K, Kawatani Y, Shibata T, Nukiwa T, Aburatani H *et al.* (2012) Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming *Cancer Cell* 22:66–79

Saldanha G, Potter L, DaForno P, Pringle JH (2006) Cutaneous melanoma subtypes show different BRAF and NRAS mutation frequencies. *Clin Cancer Res* 12:4499–505

Sekhar KR, Yan XX, Freeman ML (2002) Nrf2 degradation by the ubiquitin proteasome pathway is inhibited by KIAA0132, the human homolog to INrf2. *Oncogene* 21: 6829–34

Shibata T, Kokubu A, Gotoh M, Ojima H, Ohta T, Yamamoto M *et al.* (2008) Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. *Gastroenterology* 135(1358-68):e4

Taguchi K, Motohashi H, Yamamoto M (2011) Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells* 16:123–40

Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S *et al.* (2008) From the cover: discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc Natl Acad Sci USA* 105:3041–6

Zhang WB, Wang Z, Shu F, YH Jin, HY Liu, QJ Wang *et al.* (2010) Activation of AMP-activated protein kinase by temozolomide contributes to apoptosis in glioblastoma cells via p53 activation and mTORC1 inhibition. *J Biol Chem* 285:40461–71

# The New Aryl Hydrocarbon Receptor Antagonist E/Z-2-Benzylindene-5,6-Dimethoxy-3,3-Dimethylindan-1-One Protects against UVB-Induced Signal Transduction

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## TO THE EDITOR

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polycyclic aromatic hydrocarbons, and related environmental contaminants (Abel and Haarmann-Stemmann, 2010). The unligated AhR is trapped in a cytosolic multiprotein complex, which rapidly dissociates upon ligand binding. Subsequently, the AhR shuttles into the nucleus, dimerizes with ARNT, and binds to xenobiotic-responsive elements (XREs) in the promoter of target genes, e.g., encoding cytochrome P450 (CYP) 1 monooxygenases, to enforce transcription (Abel and Haarmann-Stemmann, 2010). Furthermore, AhR-triggered activation of c-src tyrosine kinase stimulates EGFR and downstream mitogen-activated protein kinase signaling, resulting in the induction of XRE-independent genes, such as cyclooxygenase-2 (COX-2; Abel and Haarmann-Stemmann, 2010). We have previously shown that the AhR in keratinocytes is not only activated by anthropogenic chemicals but also by UVB irradiation, which leads to the intracellular formation of the tryptophan photoproduct and high-affinity AhR ligand 6-formylindolo[3,2-*b*]carbazole (FICZ; Rannug *et al.*, 1995; Fritsche *et al.*, 2007). Indeed, UVB exposure enhances AhR/XRE binding (Supplementary Figure 1 online) and accompanied CYP1A1/1B1 expression (Katiyar *et al.*, 2000), as well as XRE-independent COX-2 expression (Fritsche *et al.*, 2007).

Because (i) overexpression of a constitutively active AhR causes inflammatory skin lesions (Tauchi *et al.*, 2005), (ii) an increase in CYP activity leads to reactive oxygen species formation (Puntarulo and Cederbaum, 1998), (iii) CYP1 enzymes are critical for chemical-induced skin carcinogenesis (Shimizu *et al.*, 2000), and (iv) COX-2 is involved in UV-induced inflammation and carcinogenesis (Elmets *et al.*, 2010), it was postulated that a transient inhibition of AhR may protect human skin against the detrimental effects of UVB irradiation (Agostinis *et al.*, 2007; Haarmann-Stemmann *et al.*, 2012). Moreover, we have shown that the expression of matrix metalloproteinase-1 (MMP-1), which is critically involved in extrinsic skin aging, is upregulated in an AhR-dependent manner in tobacco smoke extract–exposed keratinocytes (Ono *et al.*, 2013). Therefore, we decided to develop an AHR antagonist that is suitable for topical UV-protection. We screened a library of compounds that possess the structural prerequisites to

*Abbreviations: AhR, aryl hydrocarbon receptor; BDDI, E/Z-2-benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one; COX-2, cyclooxygenase-2; CYP, cytochrome P450; EROD, 7-O-ethoxyresorufin-deethylase; FICZ, 6-formylindolo[3,2-b]carbazole; MMP-1, matrix metalloproteinase-1; MNF, 3′-methoxy-4′-nitroflavone; NHEK, normal human epidermal keratinocyte; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE, xenobiotic-responsive element*

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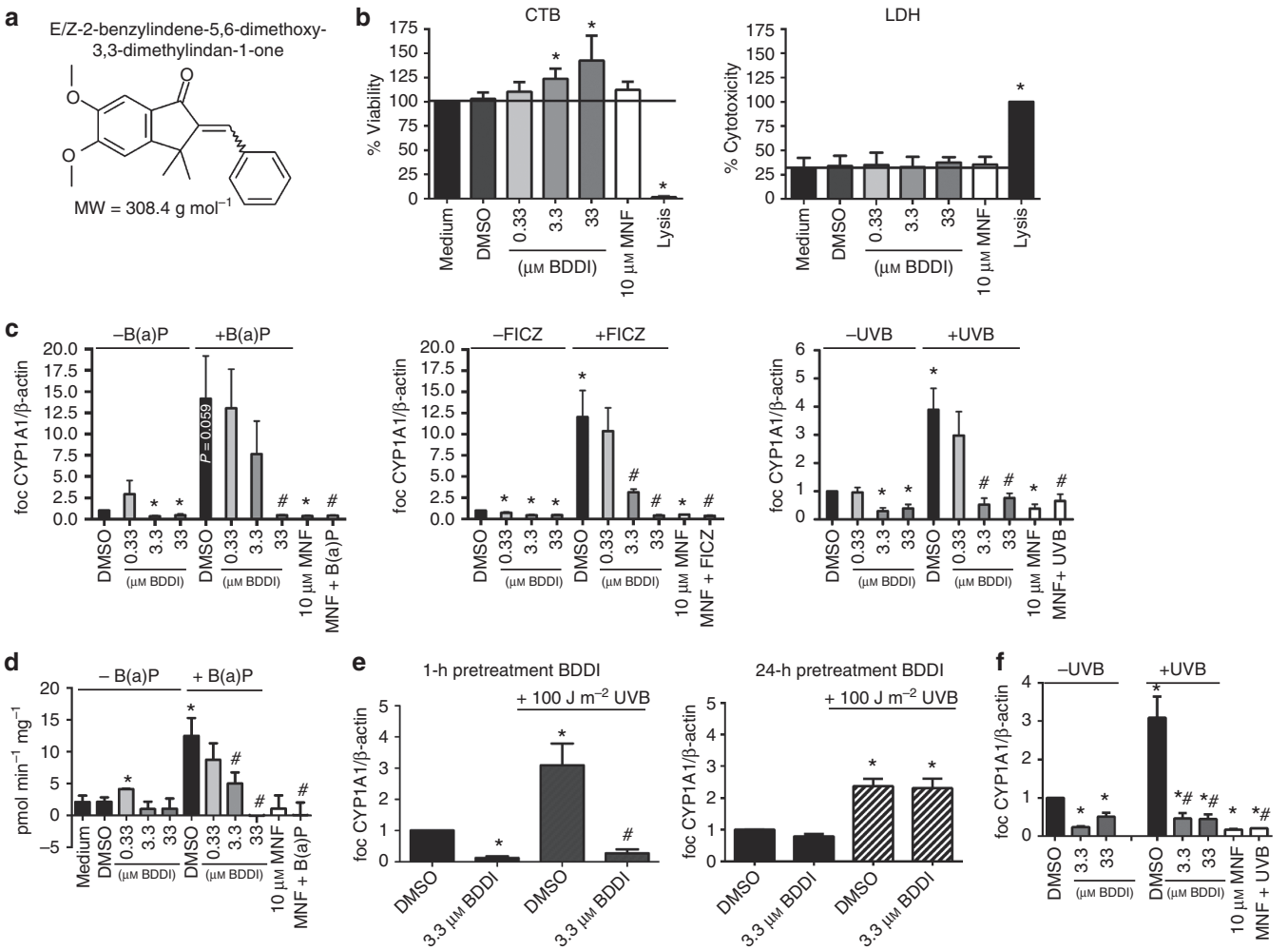


**Figure 1. Characterization of antagonistic capacities of E/Z-2-benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one (BDDI) in normal human epidermal keratinocytes (NHEKs).** (**a**) Chemical structure of BDDI. (**b**) Effect of BDDI on cell viability (CellTiter Blue; CTB) and cytotoxicity (lactate dehydrogenase; LDH). The black line marks the respective control level. (**c**) Effect of BDDI on B(a)P (250 nM for 4 hours)-, 6-formylindolo[3,2-*b*]carbazole (FICZ; 100 nM for 4 hours)-, and UVB (100 J m$^{-2}$ for 8 hours)-induced CYP1A1 mRNA expression (quantitative reverse transcription-PCR data). (**d**) Effect of BDDI on 7-*O*-ethoxyresorufin-deethylase (EROD) activity induced by 24 hours B(a)P shown in activity as pmol min$^{-1}$ mg$^{-1}$. (**e**) Transient effect of BDDI pretreatment (1 and 24 hours) on CYP1A1 mRNA expression induced by 100 J m$^{-2}$ UVB. (**f**) NHEKs were irradiated with 100 J m$^{-2}$ UVB and directly after irradiation treated with 3.3 and 33 µM BDDI. After 8 hours, RNA was isolated, and CYP1A1 transcription was analyzed. Each graph represents mean ± SEM of three independent experiments; **P*<0.05 versus medium/DMSO control; $^{\#}$*P*<0.05 versus treatment (B(a)P, FICZ, or UVB, respectively).

interact with AhR and identified E/Z-2-benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one (BDDI; Figure 1a) as the most promising candidate.

In concentrations from 0.33 to 33 µM, BDDI did not negatively affect cell viability or cause cytotoxicity in normal human epidermal keratinocytes (NHEKs; Figure 1b; for description of methods see Supplementary Material online). It is noteworthy that exposure to higher concentrations of BDDI enhanced cell viability (Figure 1b) without increasing the proliferation rate (data not shown). Exposure of NHEKs to 0.33 to 33 µM BDDI or 10 µM of the specific AhR inhibitor 3′-methoxy-4′-nitroflavone (MNF; Lu *et al.*, 1995) resulted in a concentration-dependent decrease of basal CYP1A1 expression (Figure 1c). AhR activation by 10nM FICZ or 250 nM benzo(a)pyrene [B(a)P] resulted in 12- to 14-fold induction of CYP1A1 transcription after 4 h, whereas irradiation with 100 J m$^{-2}$ UVB led to a fourfold increase in CYP1A1 expression after 8 hours (Figure 1c). Pretreatment with 10 µM MNF or 33 µM BDDI significantly attenuated CYP1A1 induction. Interestingly, a dose of 3.3 µM BDDI was sufficient to repress UVB- and FICZ-stimulated, but not B(a)P-induced, CYP1A1 expression, which was probably due to the different half-lives of the inducing agents (Figure 1c). To confirm the inhibitory effect of BDDI on CYP1A1, we treated NHEKs for 24 h with 250 nM B(a)P alone or in combination with BDDI and measured CYP1A-mediated 7-*O*-ethoxyresorufin-deethylase (EROD) activities. A 1 hour pretreatment with 0.33 to 33 µM BDDI resulted in a dose-dependent decline of B(a)P-induced EROD activity (Figure 1d), thereby confirming the AhR antagonistic properties of BDDI. Importantly, BDDI only transiently represses AhR function. Whereas a BDDI pretreatment for 1 hour attenuated UVB-mediated CYP1A1 induction

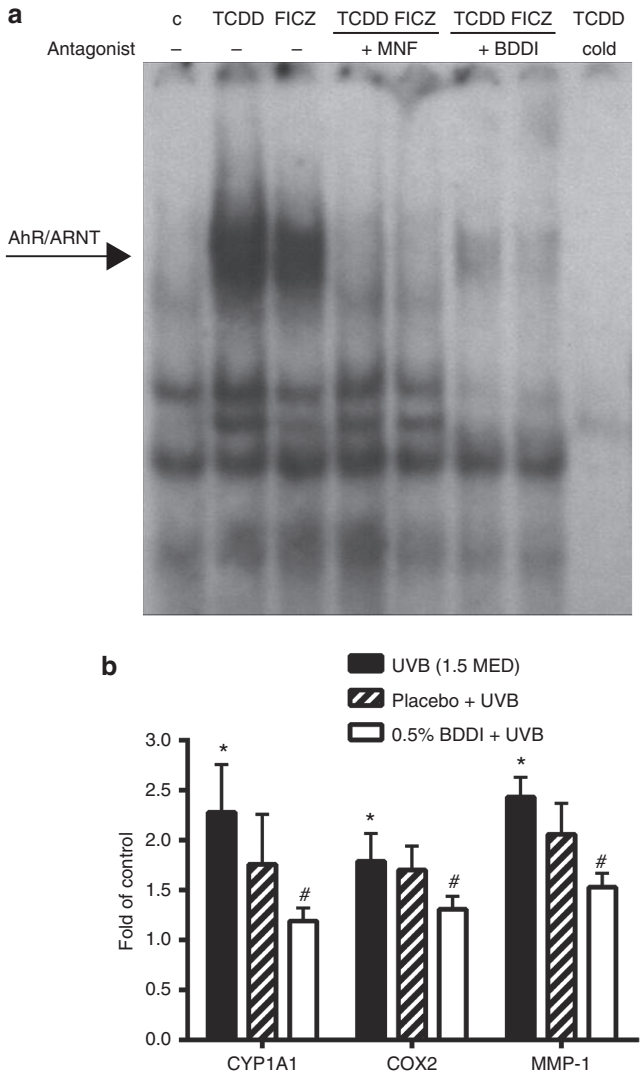


**Figure 2. BDDI disturbs XRE binding of aryl hydrocarbon receptor (AhR)/ARNT and represses UVB-induced gene expression in a human *in vivo* study.** (**a**) HaCaT keratinocytes were treated with 10 nM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 100 nM 6-formylindolo[3,2-*b*]carbazole (FICZ), 10 μM 3′-methoxy-4′-nitroflavone (MNF), and/or 3.3 μM BDDI for 90 minutes before isolation of nuclear extracts and were hybridized with a xenobiotic-responsive element (XRE) consensus oligonucleotide. A representative electrophoretic mobility shift assay of three independent experiments is shown. (**b**) Volunteers were pretreated with 0.5% BDDI or placebo for 4 days every day followed by UVB irradiation (1.5 minimal erythema dose, MED) on day 4. Twenty-four hours after irradiation, 4-mm skin biopsies were taken, RNA was isolated and reverse transcribed, and mRNA expression of CYP1A1, cyclooxygenase-2, and matrix metalloproteinase-1 was measured. Gene expression in sham-irradiated, untreated control skin were arbitrarily set as 1; $n = 10$, mean ± SEM, $^{*}P < 0.001$ versus unirradiated control, $^{\#}P < 0.001$ versus UVB (1.5 MED).

in NHEKs, a pretreatment for 24 hours was not effective (Figure 1e). BDDI treatment of NHEKs directly after irradiation also decreased UVB-mediated CYP1A1 induction, providing evidence that BDDI does not act as a UVB-filter (Figure 1f).

To elucidate the mode of action of BDDI, we performed an electrophoretic mobility shift assay (EMSA) that is well established to detect a direct binding of AhR/ARNT to a XRE consensus oligonucleotide (Denison *et al.*, 1988; Vogel *et al.*, 2004). Upon exposure of human HaCaT keratinocytes to 10 nM TCDD or 100 nM FICZ, we observed a strong binding of the AhR/ARNT complex to its DNA target motif (Figure 2a). Co-exposure of HaCaT cells to 3.3 μM BDDI or 10 μM MNF clearly blocked TCDD- and FICZ-triggered AhR/XRE binding (Figure 2a), providing evidence that BDDI acts as a true competitive AhR antagonist.

To investigate whether BDDI is suitable for UV-protection of human skin, we treated defined skin areas of 10 healthy volunteers once daily on 4 consecutive days with a formulation containing 0.5% BDDI or a placebo formulation (Figure 2b). On day 4, 2 hours after the application of the substances, volunteers were irradiated with 1.5 MED (minimal erythema dose) UVB, and 24 hours later skin biopsies were taken. Quantitative expression analyses revealed a significantly increased expression of CYP1A1, COX-2, and MMP-1 in UVB-irradiated compared with sham-irradiated skin. Topical application of BDDI, but not the placebo formulation, significantly reduced the UVB-induced expression of all these genes, indicating that BDDI penetrates human skin and blocks AhR-dependent signaling. This experiment also revealed that the AhR is activated upon UVB irradiation in human skin *in vivo*. Importantly, the erythema response of the volunteers was not significantly affected during the study.

As CYP1A1, COX-2, and MMP-1 are critically involved in cutaneous inflammatory diseases, skin cancer, and skin aging, we propose that the topical application of this chemical inhibitor presents a promising strategy to protect human skin against UVB-induced damage. In contrast to MNF (structural safety alert), BDDI has the clinical advantage of being suitable for dermal applications in humans. Our *in vitro* data further indicate that BDDI may protect against the adverse effects of polycyclic aromatic hydrocarbons, which are frequently found on airborne particulate matter (Vierkotter *et al.*, 2010). Finally, BDDI may serve as a tool to study the involvement of AhR signaling in human skin (patho)physiology.

## CONFLICT OF INTEREST

JK serves as scientific consultant for Symrise GmbH & Co. KG, Holzminden, Germany.

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## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

## REFERENCES

Abel J, Haarmann-Stemmann T (2010) An introduction to the molecular basics of aryl hydrocarbon receptor biology. *Biol Chem* 391:1235–48

Agostinis P, Garmyn M, Van Laethem A (2007) The aryl hydrocarbon receptor: an illuminating effector of the UVB response. *Sci STKE* 2007:e49

Denison MS, Fisher JM, Whitlock JP Jr. (1988) The DNA recognition site for the dioxin-Ah receptor complex. Nucleotide sequence and functional analysis. *J Biol Chem* 263: 17221–4

Elmets CA, Viner JL, Pentland AP *et al.* (2010) Chemoprevention of nonmelanoma skin cancer with celecoxib: a randomized, double-blind, placebo-controlled trial. *J Natl Cancer Institute* 102:1835–44

Fritsche E, Schafer C, Calles C *et al.* (2007) Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. *Proc Natl Acad Sci USA* 104:8851–6

Haarmann-Stemmann T, Abel J, Fritsche E *et al.* (2012) The AhR-Nrf2 pathway in keratinocytes: on the road to chemoprevention? *J Invest Dermatol* 132:7–9

Katiyar SK, Matsui MS, Mukhtar H (2000) Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. *J Invest Dermatol* 114:328–33

Lu YF, Santostefano M, Cunningham BD *et al.* (1995) Identification of 3′-methoxy-4′-nitroflavone as a pure aryl hydrocarbon (Ah) receptor antagonist and evidence for more than one form of the nuclear Ah receptor in MCF-7 human breast cancer cells. *Arch Biochem Biophys* 316: 470–7

Ono Y, Torii K, Fritsche E *et al.* (2013) Role of the aryl hydrocarbon receptor in tobacco smoke extract-induced matrix metalloproteinase-1 expression. *Exp Dermatol* 22(5):349–53

Puntarulo S, Cederbaum AI (1998) Production of reactive oxygen species by microsomes enriched in specific human cytochrome P450 enzymes. *Free Radic Biol Med* 24:1324–30

Rannug U, Rannug A, Sjoberg U *et al.* (1995) Structure elucidation of two tryptophan-derived, high affinity Ah receptor ligands. *Chem Biol* 2:841–5

Shimizu Y, Nakatsuru Y, Ichinose M *et al.* (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA* 97:779–82

Tauchi M, Hida A, Negishi T *et al.* (2005) Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions. *Mol Cell Biol* 25:9360–8

Vierkotter A, Schikowski T, Ranft U *et al.* (2010) Airborne particle exposure and extrinsic skin aging. *J Invest Dermatol* 130:2719–26

Vogel CF, Sciullo E, Park S *et al.* (2004) Dioxin increases C/EBPbeta transcription by activating cAMP/protein kinase A. *J Biol Chem* 279:8886–94

# A Bio-Mimetic Approach to DNA Photoprotection

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## TO THE EDITOR

Exposure to solar UVR continues to be a major contributing factor in the increase in the incidence of skin cancer. Mutagenic photoproducts formed in the DNA of UV-exposed cells, specifically due to absorption of radiation in the UVB range of 280–320 nm, include cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6–4) photoproducts (Rastogi *et al.*, 2010). The CPDs are the major contributors to mutations in mammals, as they are repaired less quickly than (6–4) photoproducts. Recent studies suggest that some of the existing UV-filtering agents can form harmful photodegradation products (Hanson *et al.*, 2006; Mosley *et al.*, 2007; Burnett and Wang, 2011). Therefore, there is a pressing need for new strategies of photoprotection to reduce the detrimental effects of UVR. To this end, we adopted a bio-mimetic approach for photoprotection using acyclothymidine dinucleosides (aTds).

It has been shown that topical application of thymidine dinucleotides resulted in DNA photoprotection (Goukassian *et al.*, 2004, 2012; Arad *et al.*, 2008). The aTds eliminate the complex and unstable phosphate-linked diribofuranosyl moieties in oligonucleotides and are inexpensive to prepare (Figure 1a). In addition, they retain the ability to offer photoprotection by mimicking the vulnerable adjacent thymidine sequences present in DNA molecules.

We have recently reported that aTd protects plasmid and cellular DNA (*in vitro*) from UVR while undergoing a photocatalyzed [2 + 2] cycloaddition to form UV non-cytotoxic cyclobutane dimers (Raza *et al.*, 2013). These non-cytotoxic mimetics of naturally occurring DNA dithymidine sequences have UV adsorption spectra comparable to native DNA thymidine and are similar to the naturally occurring excised DNA thymine dimers. We have now examined the efficacy of aTd to reduce the incidence of cellular DNA thymine dimer formation after exposure to UVB irradiation in three separate experiments using the SKH1-E hairless mouse model (Crl:SKH1-*Hr* $^{hr}$ strain code 477, Charles River Laboratories International, Wilmington, MA) and human abdominal skin explants. For all studies, detailed methodology can be found in accompanying Supplementary Information online.

## ACUTE UVB EXPOSURE

Dorsal skin of immunocompetent, hairless SKH1-E mice and human

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