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Comparative analysis of the phototoxicity induced by BRAF inhibitors and alleviation through antioxidants

Running title: Phototoxicity induced by BRAF inhibitors

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ABSTRACT

Background: Small molecules tackling mutated BRAF (BRAFi) are an important mainstay of targeted therapy in a variety of cancers including melanoma. Albeit commonly reported as side effect, the phototoxic potential of many BRAFi is poorly characterized. In this study, we evaluated the phototoxicity of 17 distinct agents and investigated whether BRAFi-induced phototoxicity can be alleviated by antioxidants.

Methods: The ultraviolet (UV) light absorbance of 17 BRAFi was determined. Their phototoxic potential was investigated independently with a reactive oxygen species (ROS) and the 3T3 neutral red uptake (NRU) assay *in vitro*. To test for a possible phototoxicity alleviation by antioxidants, vitamin C, vitamin E phosphate, trolox, and glutathione (GSH) were added to the 3T3 assay of selected inhibitors.

Results: The highest cumulative absorbance for both UVA and UVB was detected for vemurafenib. The formation of ROS was more pronounced for all compounds after irradiation with UVA than with UVB. In the 3T3 NRU assay, 8 agents were classified as phototoxic, including vemurafenib, dabrafenib, and encorafenib. There was a significant correlation between the formation of singlet oxygen ($p = 0.026$) and superoxide anion ($p < 0.001$) and the phototoxicity observed in the 3T3 NRU assay. The phototoxicity of vemurafenib was fully rescued in the 3T3 NRU assay after GSH was added at different concentrations.

Conclusion: Our study confirms that most of the BRAF inhibitors exhibited a considerable phototoxic potential, predominantly after exposure to UVA. GSH may help treat and prevent the phototoxicity induced by vemurafenib.

Keywords: protein kinase inhibitor, BRAF, phototoxicity, antioxidant, targeted therapy

SUMMARY STATEMENT

Kinase inhibitors are increasingly being used as targeted therapy in medical oncology. However, some agents are accompanied by high phototoxicity. Here, we performed a comparative analysis of 17 kinase inhibitors and investigated whether their phototoxic potential can be alleviated through antioxidants with two independent assays *in vitro*. Our study confirms that most of the agents investigated exhibited a considerable phototoxic potential, predominantly after exposure to UVA. Interestingly, the phototoxic potential of the BRAF inhibitor vemurafenib decreased after the antioxidant glutathione was added. Thus, glutathione may help to treat and prevent the phototoxicity induced by vemurafenib.

INTRODUCTION

The identification of somatic mutations of the *BRAF* gene has paved the way for targeted therapy with small molecules in a variety of cancer entities in recent years.[1] Activating genetic alterations of codons encoding for the kinase domain of BRAF result in constitutive oncogenic signalling through the Ras-Raf-MEK-ERK mitogen-activated protein kinase (MAPK) pathway, leading to cell proliferation and, ultimately, tumor growth.[2] Mutations of *BRAF* have been found in more than 66% of human cancers and are suspected to be present in an even wider range of malignancies at a lower frequency.[3] Encouraged by these observations, major pharmaceutical efforts have been made at high pace to develop small molecules targeting mutant BRAF. As the target protein with and without the mutation of interest could be structurally elucidated in its active and inactive conformation via crystallography, a hitherto never utilized drug design strategy was employed, namely fragment-based lead discovery. Here, smaller well-binding structures are joined covalently to form a superiorly target-binding drug candidate.[4] As a result, the BRAF inhibitors (BRAFi) vemurafenib and dabrafenib were developed and approved by the FDA for the treatment of metastatic or unresectable melanoma in 2011 and 2013, respectively, showing substantial survival benefits compared to chemotherapy.[5,6] In 2018, a third inhibitor, encorafenib, has been approved in combination with the MEK inhibitor, binimetinib.[7] All substances selectively bind to and inhibit the active-state BRAF kinase, with most BRAFi sharing common structural motifs: the A ring binding in the nucleobase-binding pocket, the B ring as a sterically important stiff core, the BC linker (salt bridge linker) for ionic interactions, and the lipophilic C ring.[8] As the A ring resembles

the aromatic, bicyclic adenine-moiety of the native substrate ATP, most inhibitors rely on a mono- or bicyclic, heavily substituted aromatic structure for strong binding characteristics. For this reason, most inhibitors exhibit strong UVA absorbance which is a prerequisite for UVA-induced phototoxicity. This cutaneous adverse event is well known for vemurafenib, while the phototoxic potential of dabrafenib and encorafenib is much lower in pivotal trials.[9-13] However, the phototoxic potential of other BRAFi is poorly characterized and has not been analyzed in a systematic approach yet. Therefore, in this study we comparatively evaluated the phototoxicity of 17 distinct BRAFi or multikinase inhibitors and tested whether phototoxicity can be reduced by antioxidants *in vitro*.

MATERIALS AND METHODS

Chemicals

All BRAF inhibitors, including AZ628, CEP-32496, dabrafenib, encorafenib, GDC-0879, GW5074, LY3009120, MLN2480, NVP-BHG712, PLX-4720, RAF265, RO5126766, SB590885, sorafenib tosylate, TAK-632, vemurafenib, and ZM336372 were purchased from Selleck Chemicals LLC (Houston, TX/USA). Concentrations of the inhibitor stock solutions in DMSO were normalized to the least soluble compound (sorafenib tosylate) at 7.85 mM. Control compounds (chlorpromazine, quinine, and sulisobenzone), antioxidants such as ascorbic acid (vitamin C), (±)-α-tocopherol phosphate disodium salt (vitamin E phosphate), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox, vitamin E analog), and glutathione (GSH), and additional chemicals were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions were stored frozen and protected from light. Ultraviolet-visible spectra of the test compounds were determined with an IMPLEN NanoPhotometer™ (Munich, Germany).

Irradiation conditions

Irradiance is defined as the intensity of UV or visible light incident on a surface, measured in W/m^2 or mW/cm^2. Cells in 96-well plates were illuminated with either UVA light by F8T5/PUVA fluorescent tubes (main emission 320-410 nm, maximum 351 nm; Sylvania, USA) or UVB light by broadband TL 20W/12 RS ultraviolet-B fluorescent tubes (main emission 290-320 nm, maximum 302 nm; Philips, Germany). The irradiation tests were performed at 25 °C with an irradiance of approximately 4.1 mW/cm^2 (UVA) or

0.41 mW/cm^2 (UVB) which was tested prior to each experiment.

Cell culture methods

Sterile cell culture plasticware was obtained from Greiner BioOne (Frickenhausen, Germany) and Sarstedt (Nümbrecht, Germany), while chemicals and media were acquired from Gibco/Invitrogen (NY/USA) and Sigma-Aldrich (Steinheim, Germany). Mouse BALB/c embryo 3T3 clone A31 fibroblasts were purchased from the European collection of cell cultures (ECACC) *via* Sigma-Aldrich. The cells were used between passage numbers 30 to 40. All illumination procedures were performed in a photobiology laboratory with no measurable UV light levels (Waldmann, Germany). 3T3 cells were maintained in DMEM (Dulbecco's Modified Eagle Medium, 4.5 g/l glucose) supplemented with 10% fetal bovine serum (FBS), 4 mM stable glutamine, penicillin (100 IU) and streptomycin (100 µg/ml), and humidified incubation at 37 °C with 5% CO_2. Cells were subcultured every 3 to 4 days.

Reactive oxygen species assay

The reactive oxygen species (ROS) assay was developed to test for the generation of a reactive species from chemicals following absorption of UV-visible light, as a key determinant of chemicals for causing phototoxic reactions.[14] Based on the OECD/OCDE guidelines for the ROS assay tests, stock solutions of all chemicals were prepared at 20 mM in sodium phosphate buffer (NaPB, pH 7.4) or at 7.85 mM in DMSO. 0.2 mM p-nitrosodimethylaniline (RNO) were prepared by dissolving 3 mg of RNO in 100 mL of 20 mM NaPB. 13.6 mg of imidazole were dissolved in 10 mL of 20 mM NaPB and the 2×10^{-2} M imidazole solution was diluted 100 times with 20 mM NaPB, forming a 20 mM imidazole compound. 0.4 mM nitroblue tetrazolium chloride (NBT) were prepared by dissolving 32.7 mg of NBT in 100 mL of 20 mM NaPB. Quinine hydrochloride (QUI) and sulisobenzone (SIB) were used as positive and negative controls, respectively.[15]

The test procedure for this assay included a 1.5 mL micro tube and a plastic clear flat bottomed 96-well microplate. The reaction mixtures were prepared by vortex mixing under UV-cut illumination. For each reaction mixture, triplicates of 200 µl per well were transferred into a 96-well plate and solubility and coloration were checked microscopically at 100-fold magnification. After shaking the plate for 5 seconds, baseline absorbance at

440 nm (A_{440}) for SO and 560 nm (A_{560}) for SA was measured prior to UV exposure. The plate was irradiated with a UVA simulator for 1 h, and A_{440} and A_{560} were measured again. Based on the assay protocol, SO was determined as a result of bleaching of p-nitrosodimethylaniline by oxidized imidazole. The measurement of SA was made upon the reduction of nitroblue tetrazolium:[16]

SO generation = 1000 x [$A_{440(\text{no UVA})}$ - $A_{440(\text{UVA})}$ – ($\text{solvent}_{(\text{no UVA})}$ – $\text{solvent}_{(\text{UVA})}$]

SA generation = 1000 x [$A_{560(\text{no UVA})}$ – $A_{560(\text{UVA})}$ – ($\text{solvent}_{(\text{UVA})}$ – $\text{solvent}_{(\text{UVA})}$]

***In vitro* 3T3 NRU phototoxicity test**

Identification by this test increases the likelyhood of substances to be phototoxic *in vivo* after systemic or topical application.[17] Phototoxicity of the BRAFi was determined according to the OECD/OCDE 432 guideline with minor modifications.[18] For the experiments, 3T3 cells were seeded into 96-well plates at a density of 5×10^4 cells per well. The outer wells of each plate were left empty. All test compounds were checked for degradation by determination of the UV-visible spectra (IMPLEN NanoPhotometer™, Munich, Germany) prior to each experiment. After 24 h, the cells were washed with phosphate-buffered saline (PBS) and incubated with the inhibitors at different concentrations (100 µM, 10 µM, 1 µM, 100 nM, 10 nM, 0 nM or 100 µM, 31.6 µM, 10 µM, 3.16 µM, 1 µM, 0 µM) in Earle's balanced salt solution (EBSS) with low bicarbonate (0.085%) for 1 h in the dark at 37 °C. Chlorpromazine (CPZ) and quinine (QUI) were used as positive controls, sulisobenzone (SIB) as negative control for UV phototoxicity. The percentage of solvents in the experiments did not exceed 1.2% (v/v) at the highest concentrations tested.

After incubation of two identical 96-well plates, one was exposed to either UVA (total dose: 5 J/cm^2) or UVB (total dose: 20 mJ/cm^2) light and the other one was covered in lightproof aluminum foil and incubated under the UV lamp as well. Subsequently, the cells were washed with PBS and incubated in DMEM supplemented with FBS at 37°C overnight. On the following day, cells were washed with PBS and incubated in DMEM without FBS containing 50 µg/ml neutral red (NR) dye at 37°C for 2 h. Cells were washed with PBS and blotted to remove buffer remains. Precisely, 150 µl desorb solution (50% ethanol v/v, 1% acetic acid (v/v)) were added per well and the plate was incubated at room temperature for 10 min with gentle shaking. The absorbance of the resulting

homogeneously pink solution was measured without a lid at 540 nm in a plate reader (Spectra MR, Dynex Technologies, Germany). The outer wells of each plate were used as reference.

Phototoxicity alleviation through antioxidants

To test for an effect on the BRAFi-mediated toxicity, antioxidants were added to the 3T3 NRU phototoxicity test of selected inhibitors. The concentrations of the tested antioxidants were 100 µM, 10 µM, 1 µM for vitamin C, vitamin E phosphate, and trolox, and 10 mM, 1 mM, and 100 µM for GSH. The concentrations of the BRAFi vemurafenib, dabrafenib and encorafenib were 3.16 µM, 1 µM and 10 µM, respectively.

Data evaluation

Each data point was at least measured in duplicate and in two independent experiments. To visualize the data, dose-response curves were created, showing relative viability against the BRAFi concentration applied at a fixed irradiation dose (no UV, UVA, UVB). The IC_{50} was defined as the concentration reducing cell viability to 50% compared with that of untreated control cultures. IC_{50} values were calculated *via* curve fits using GraphPad Prism® 5 for Windows. Phototoxicity was calculated according to the OECD 432 guideline, where the "photo-irritation-factor" (PIF; ratio of IC_{50noUV} to IC_{50UV}) was used to estimate the risk.[18] Based on the validation study, a PIF value less than 2 predicts no phototoxicity, a value between 2 and 5 probable phototoxicity, and more than 5 phototoxicity.[17] In some cases, not both IC_{50} could be determined, so that the published *ad hoc* rules were applied (prediction model 1): First, if only one IC_{50} can be measured, the other value is replaced by the highest concentration tested; the chemical is considered phototoxic if the ratio is greater than 1. Second, if no IC_{50} can be measured, the chemical is considered non-phototoxic.[19] The correlation between the formation of SO or SA in the ROS assay and phototoxicity observed in the 3T3 NRU assay was calculated with Pearson's correlation. Differences of phototoxcity after the addition of antioxidants were compared with the student's t-test. A two-sided p-value was calculated in all cases and values of $p<0.05$ considered as statistically significant.

RESULTS

UV spectral analysis of BRAF kinase inhibitors

Initially, the UV absorbance spectrum of the 17 Raf inhibitors was determined. All inhibitors showed a certain amount of UVA (λ_{UVA} = 315 to 410 nm) and UVB light (λ_{UVB} = 280 to 315 nm) absorbance (Fig. 1A). The substance with the highest absorption of UVA light was GW5074. The area under the curve (AUC) of this compound was set to 1 for UVA (Fig. 1C). It was closely followed by SB590885 (relative absorption 0.8). Out of the 4 inhibitors approved for cancer treatment, vemurafenib and dabrafenib appeared to have the highest rates of UVA light absorptions (relative absorptions 0.3), with encorafenib following at a slightly lower rate (relative absorption 0.2). The only RAF kinase inhibitor exhibiting almost no UVA light absorption was sorafenib tosylate (relative absorption 0) (Fig. 1B).

The highest absorbance of UVB was observed for NVP-BHG712 whose AUC and relative absorption was set to 1 (Fig. 1C). Compared to its absorbance of UVA, sorafenib tosylate displayed a much higher absorption of UVB (relative absorption 0.5) in our spectral analysis. Interestingly, the highest amount of UVB absorption amongst the approved BRAFi was recorded again for vemurafenib (relative absorption 0.8) and the lowest for encorafenib (relative absorption 0.2) (Fig. 1B). The absorbance curve for vemurafenib showed a peak at the border between UVB and UVA and then decreased after 340 nm (Fig. 1A+B).

***In chemico* generation of reactive oxygen species upon exposure to UVA and UVB light**

In chemico SO and SA generation induced by BRAFi upon exposure to UVA and UVB was analysed. Dabrafenib was the only chemical showing increased SA levels after UV irradiation while also leading to increased SO formation. Only 3 out of 17 compounds generated SO species following absorption of UVB light, whereas, after absorption of UVA light, 9 chemicals including encorafenib resulted in increased levels of SO. Interestingly, vemurafenib and sorafenib tosylate belonged to the 7 substances where no phototoxic response was detected (Table 1).

***In vitro* 3T3 NRU phototoxicity assay**

We compared the IC_{50} values acquired in the light and dark experiments by calculating the

PIF. Since most BRAFi showed no toxicity without irradiation in the concentrations tested, the *ad hoc* rules were applied. Six of the 17 chemicals under evaluation were classified according to the PIF values as non-phototoxic (GDC-0879, RAF265, RO5126766, SB590885, sorafenib tosylate, ZM336372) (Fig. 2C), 3 as probably phototoxic (CEP-32496, LY3009120, PLX-4720) (Fig. 2B), and 8 as phototoxic (AZ628, dabrafenib, encorafenib, GW5074, MLN2480, NVP-BHG712, TAK-632, vemurafenib) (Fig. 2A). The highest PIF_{UVA} values were calculated for dabrafenib (≥925.1), followed by TAK-632 (≥61.9), and encorafenib (≥55.7). No substantial UVB phototoxicity could be recorded as all PIF_{UVB} values were close to 1, although no threshold values have been reported in the literature (Table 2).

Phototoxicity alleviation through antioxidants

Comparing the results of the ROS and the 3T3 NRU phototoxicity assay, we detected a significant correlation between the phototoxicity observed in the 3T3 NRU assay and the formation of both SO (Pearson r = 0.5365; p=0.026) and SA (Pearson r = 0.9139; p<0.001) in the ROS assay. To test for an inhibitory effect on the BRAFi-mediated toxicity, antioxidants were added to the 3T3 NRU phototoxicity test of selected inhibitors. The phototoxicity of the approved BRAFi vemurafenib, dabrafenib, and encorafenib was analysed in the presence or absence of UVA light for different concentrations of glutathione (GSH), trolox, vitamin C and vitamin E. Of all tested antioxidants, high-dose glutathione was able to fully rescue the phototoxicity observed with vemurafenib after exposure to UVA (Fig. 3A). In contrast, the phototoxicity induced by dabrafenib and encorafenib was left unaltered after antioxidants were added at different concentrations (Fig. 3B and 3C).

DISCUSSION

Activating mutations of BRAF can be found in 40-60 % of patients suffering from advanced melanoma, leading to consistent activation of downstream signalling through the MAPK pathway.[3] This discovery has provided an important target for small-molecule drugs that have successfully been introduced in the recent years for melanoma therapy. However, patients treated with BRAFi frequently develop cutaneous adverse reactions, such as cutaneous squamous cell carcinoma, verrucal keratosis, and photosensitivity,

which can severely impact the quality of life.[20] Even though phototoxicity belongs to the most commonly reported adverse events since the introduction of the first BRAFi vemurafenib, the phototoxic potential of other inhibitors has not been systematically analysed yet.[5,21] Therefore, we tested the phototoxicity of 17 different BRAFi and investigated whether phototoxicity can be rescued by adding antioxidants *in vitro*.

The generation of reactive oxygen species following UV light irradiation can lead to oxidative damage to the cell. UVA light plays a more significant role in causing phototoxicity than other UV ranges.[22,23] This is consistent with the results of the ROS assay that was performed in this study with UVA and UVB light. Only 3 out of 17 substances induced the formation of ROS following irradiation with UVB light compared to 9 agents which generated ROS after UVA light absorption. Surprisingly, vemurafenib elicited no ROS release in our assay, although its UVA-dependent phototoxicity is well established in daily care and in the literature.[9,24] These results imply that the phototoxicity observed with vemurafenib clinically is not mediated by ROS. In contrast, we observed that dabrafenib, which in the clinical practice is considered much less phototoxic than vemurafenib, was the only substance leading to the formation of both SO and SA after UVA and UVB exposure. Thus, our results fit well with a recent study, in which vemurafenib but not dabrafenib impaired the repair of UV-induced DNA damage in keratinocytes.[25] These results suggest that the phototoxicity experienced by patients under BRAFi *in vivo* may not primarily be mediated by ROS formation and imply that also other mechanisms are likely to be involved. Above that, we conclude that the ROS assay may not accurately predict the phototoxicity which is clinically relevant and observed *in vivo*.

Another test which has proved to be predictive of acute phototoxicity effects in animals and humans *in vivo* is the 3T3 NRU phototoxicity test.[18] Overall, 8 compounds including the BRAFi approved for melanoma encorafenib, vemurafenib and dabrafenib were identified as phototoxic by this test. In accordance with the ROS assay results, the 3T3 NRU test results also confirmed the phototoxicity of dabrafenib with a considerable PIF value over 900, the highest among all test chemicals. Vemurafenib, in contrast to the ROS assay results, proved to be phototoxic in the 3T3 NRU test, whereas its PIF value (>5.634) was much lower than that of dabrafenib. Nevertheless, the results observed in our 3T3 NRU assays were in line with various *in vivo* studies, which have shown emergence of

photosensitivity during vemurafenib or dabrafenib therapy.[6,24] The reason why the phototoxicity *in vivo* of dabrafenib is lower than suggested by the ROS and the 3T3 NRU assay remains unclear. Our data support a hypothesis by Gabeff et al. that dabrafenib is a phototoxic agent *per se* but has a different triggering cut-off for a reaction to UVA radiation compared to vemurafenib. Another explanation for the difference between the high phototoxicity detected *in vitro* and low frequence of phototoxic adverse events experienced *in vivo* is the slight delay of dabrafenib studies compared to those of vemurafenib, during which patients had already received the advice for sun-protection.[22] Furthermore, the cumulative UV absorbance did not correlate well with the phototoxicity observed in the 3T3 assay for all substances. In particular, agents with high absorbance for UVB such as RAF265, RO5126766, and SB590885 were classified as non-phototoxic in the 3T3 assay. In contrast, other agents with high UVB absorbance such as NVP-BHG712 and vemurafenib were classified as phototoxic in this assay, underlining that the amount and type of absorbance did not necessarily reflect their phototoxic potential. Of the 6 test chemicals without phototoxic potential (PIF<2) in the 3T3 NRU assay, only the multikinase inhibitor sorafenib is used in the clincial routine. Our results are consistent with the assessments of other studies that have reported numerous sorafenib-associated dermatologic side effects which, however, rarely include phototoxicity.[26,27]

To alleviate the BRAFi-induced phototoxicity, we tested a panel of antioxidants as an attempt to find feasible alternatives for the management of phototoxicity.[28,29] Accordingly, the physiological antioxidants vitamin C, vitamin E, trolox and GSH were added to the 3T3 NRU phototoxicity assay with the BRAFi vemurafenib, dabrafenib, and encorafenb. Interestingly, we observed that high-dose GSH was able to fully rescue the UVA-induced phototoxicity of vemurafenib. GSH is a tripeptide best known for its role as antioxidant by neutralising ROS. However, as no induction of ROS was observed with vemurafenib, it is likely that other functions of GSH are involved. It has general cytoprotective properties and can stabilize cellular components after DNA damage.[30] Thus, our data support a model where UV-induced damage repair is impaired by vemurafenib and that this process can be alleviated by GSH, independently from the generation of ROS. Although our understanding of the protective effects of GSH on the vemurafenib-induced phototoxicity is certainly limited, substances that increase GSH may represent an interesting option to

protect patients from photoxicity also *in vivo*. Further studies are warranted to fully explore the potential of GSH in both the treatment and prevention of photoxic reactions due to vemurafenib.

CONFLICT OF INTEREST

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TABLE LEGENDS

Table 1: Analysis of *in chemico* ROS generation upon exposure to UVA and UVB light according to the cut-off values of the OECD guideline for the testing of chemicals.[15]

Table 2: Photo-Irritation-Factors (PIF; ratio of $IC50_{noUV}$ to $IC50_{UV}$) of the 17 tested Raf inhibitors upon exposure to UVA light.

FIGURE LEGENDS

Figure 1: *In chemico* analysis of UV absorption spectrum of Raf inhibitors. (A) absorbance of UVA (λ_{UVA} = 315 to 410 nm) and UVB light (λ_{UVB} = 280 to 315 nm) of 17 Raf inhibitors was determined. (B) UV absorbance of the approved inhibitors vemurafenib, dabrafenib, encorafenib, and sorafenib tosylate. (C) Relative UV absorbance of the 17 tested compounds determined by the area under the curve (AUC). The AUC of GW5074 and NVP-BHG712 was set to 1 for UVA and UVB, respectively. All other AUCs were normalized to these values.

Figure 2: *In vitro* 3T3 NRU phototoxicity assay. 3T3 mouse fibroblasts were exposed to different concentrations of inhibitors and irradiated with UVA or UVB light. A photo-irritation-factor (PIF: ratio of IC_{50}noUV to IC_{50}UV) was calculated to estimate the phototoxic potential of each test compound. Dose response curves of (A) phototoxic (PIF > 5), (B) probably phototoxic (PIF ranging from 2 to 5) and (C) not phototoxic test substances (PIF <2) are shown.

Figure 3: Efficacy of antioxidants as inhibitors of phototoxicity *in vitro*. The inhibiting effects of the antioxidants glutathione (GSH), trolox, vitamin C and vitamin E on the drug-induced phototoxicity of the BRAF kinase inhibitors vemurafenib (A), dabrafenib (B) and encorafenib (C) were analysed; *p<0.05, **p<0.01, ***p<0.001 vs. contol (+ UVA, Ø antioxidant).

Table 1: Analysis of *in chemico* ROS generation upon exposure to UVA and UVB light.

	ROS assay			
	+ UVB		+ UVA	
Inhibitor	**singlet oxygen**	**superoxide anion**	**singlet oxygen**	**superoxide anion**
AZ628	+	-	-	-
CEP-32496	-	-	-	-
Dabrafenib	+	+	+	+
Encorafenib	-	-	+	-
GDC-0879	-	-	+	-
GW5074	-	-	+	-
LY3009120	-	-	-	-
MLN2480	+	-	+	-
NVP-BHG712	-	-	+	-
PLX-4720	-	-	-	-
RAF265	-	-	+	-
RO5126766	-	-	-	-
SB590885	-	-	+	-
Sorafenib tosylate	-	-	-	-
TAK-632	-	-	+	-
Vemurafenib	-	-	-	-
ZM336372	-	-	-	-

+: ROS detectable, -: ROS not detectable.

Table 2: Photo-Irritation-Factors.

phototoxic PIF > 5		probably phototoxic PIF 2 to 5		not phototoxic PIF < 2	
AZ628	>10.004	CEP-32496	>2.91	GDC-0879	>1.08
dabrafenib	>925.069	LY3009120	>3.867	RAF265	0
encorafenib	>55.679	PLX-4720	>3.667	RO5126766	0
GW5074	>7.127			SB590885	0
MLN2480	>8.834			sorafenib tosylate	>1.021
NVP-BHG712	>11.089			ZM336372	0
TAK-632	>61.92				
vemurafenib	>5.634				

Figure 1

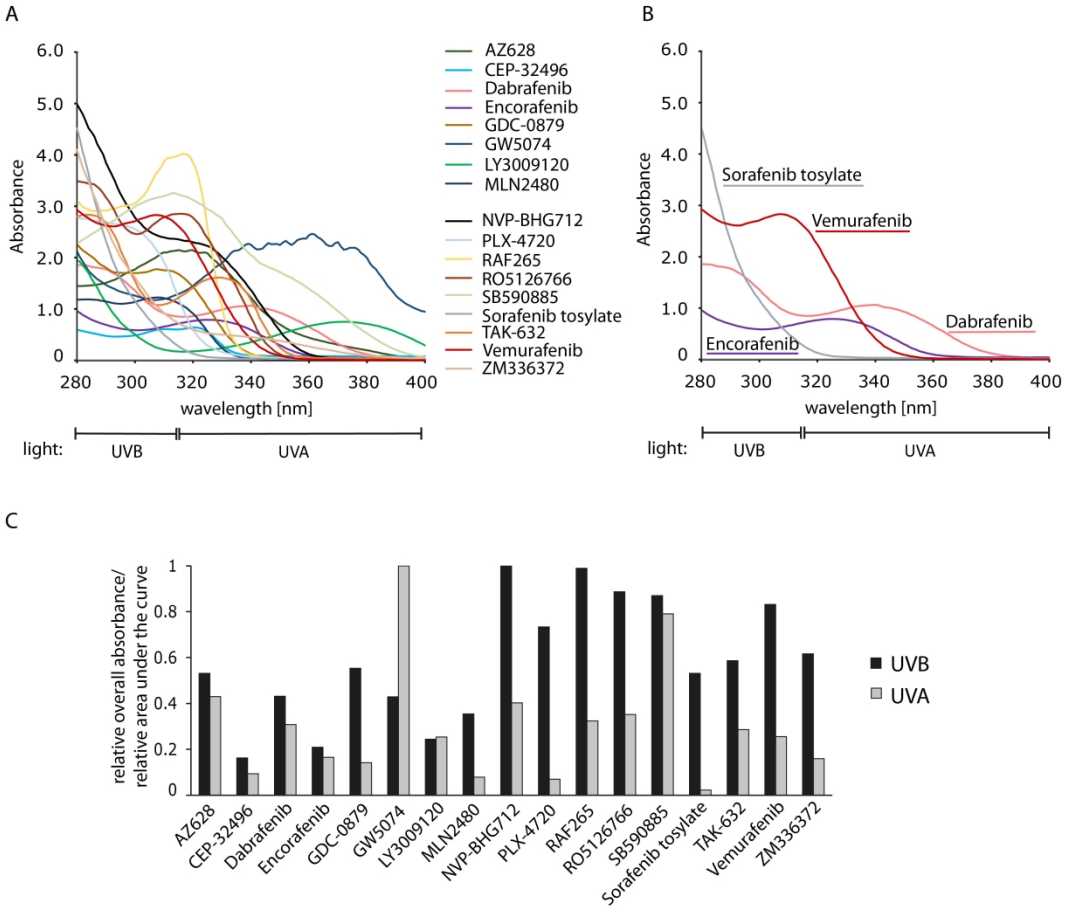

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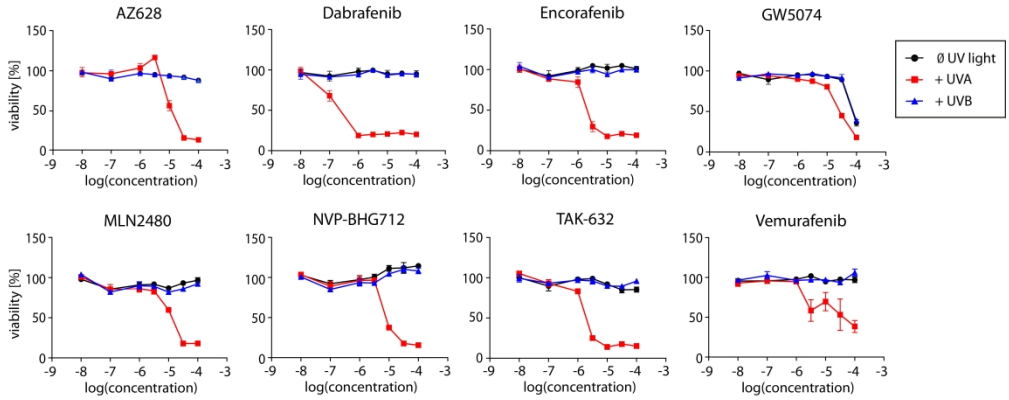
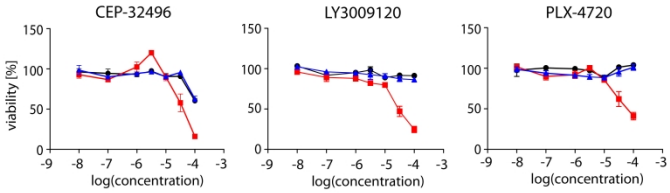
Figure 2

A phototoxic compounds



B probably phototoxic compounds



C non-phototoxic compounds

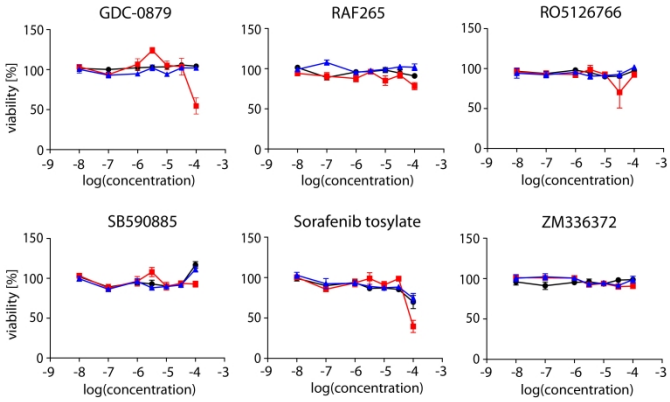


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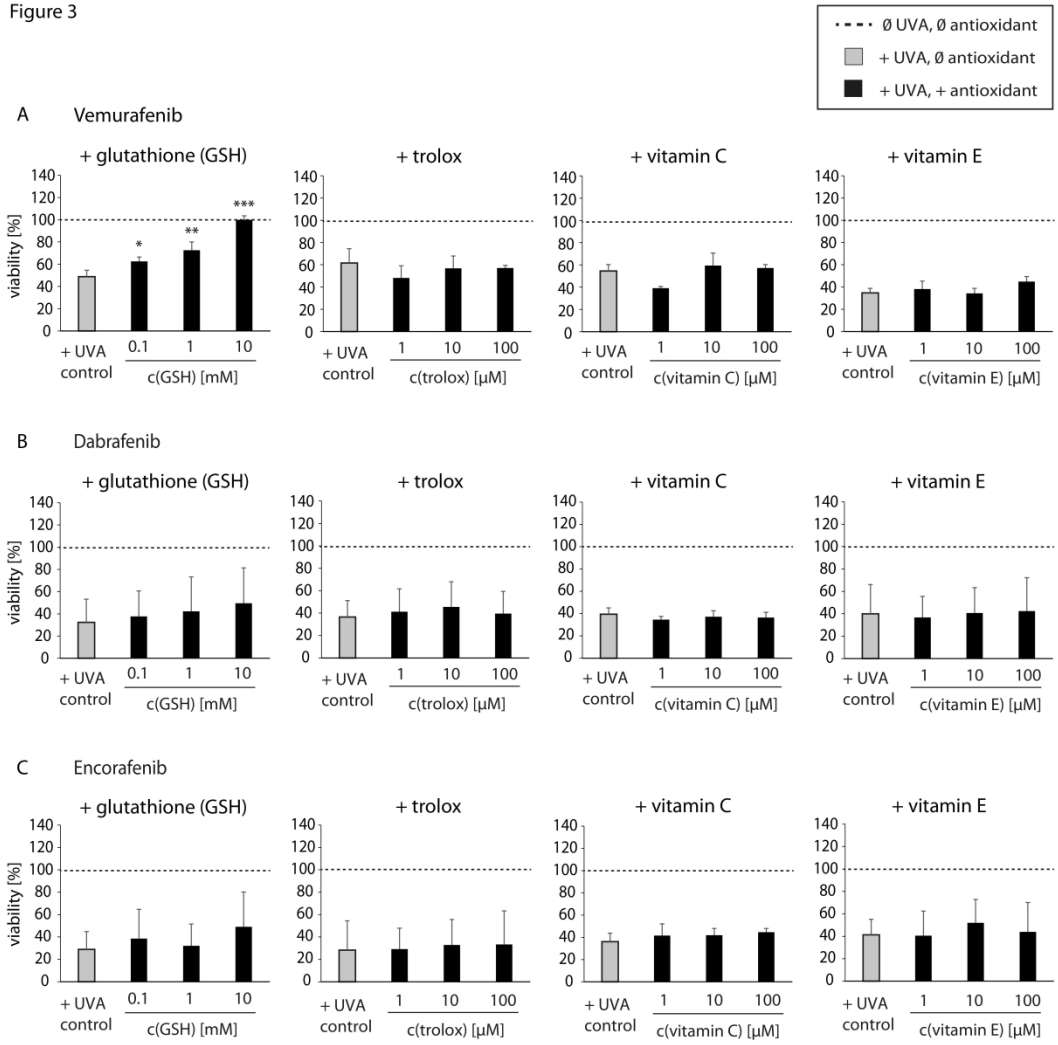
Figure 3



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