Acute Mitochondrial Inhibition by Mitogen-activated Protein Kinase/Extracellular Signal-regulated Kinase Kinase (MEK) 1/2 Inhibitors Regulates Proliferation*

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Background: Small molecule MEK1/2 inhibitors are designed to block ERK1/2-mediated signaling and inhibit proliferation.

Results: Novel instrumentation which measures cellular respiratory chain function found that four structurally distinct MEK1/2 inhibitors acutely affected mitochondrial bioenergetics.

Conclusion: The anti-mitochondrial effects of MEK1/2 inhibitors determined proliferative potential.

Significance: Impaired mitochondrial metabolism following MEK1/2 inhibition must be considered when using small molecule inhibitors to define this signaling pathway.

The Ras-MEK1/2-ERK1/2 kinase signaling pathway regulates proliferation, survival, and differentiation and, because it is often aberrant in tumors, is a popular target for small molecule inhibition. A novel metabolic analysis that measures the real-time oxidation state of NAD(H) and the hemes of the electron transport chain and oxygen consumption within intact, living cells found that structurally distinct MEK1/2 inhibitors had an immediate, dose-dependent effect on mitochondrial metabolism. The inhibitors U0126, MIIC and PD98059 caused NAD(H) reduction, heme oxidation, and decreased oxygen consumption, characteristic of complex I inhibition. PD198306, an orally active MEK1/2 inhibitor, acted as an uncoupler. Each MEK1/2 inhibitor depleted phosphorylated ERK1/2 and inhibited proliferation, but the most robust antiproliferative effects always correlated with the metabolic failure which followed mitochondrial inhibition rather than inhibition of MEK1/2. This warrants rethinking the role of ERK1/2 in proliferation and emphasizes the importance of mitochondrial function in this process.

Most cancers develop autonomy from growth factor signaling often through mutations or changes in protein expression (1). The mitogen-activated protein kinase (MAPK) cascades have been identified as key regulators of cellular proliferation, differentiation, and survival, and one of these cascades, Raf-MEK1/2-ERK1/2, has been the target of many pharmaceutical endeavors (2–7). Processes as diverse as survival following oxidant injury, steroid biosynthesis, VEGF release, neuronal development, and cell cycle progression are believed to be regulated via this cascade where the end point obtained depends not only on the phosphorylation event but also on factors such as the duration and cellular location of the signal (8–15).

Because RAS structural alterations occur in nearly 30% of all cancers and BRAF mutations have been found in up to 60% of certain cancers, small molecule inhibitors of this signaling pathway have been widely developed and tested (10, 16, 17). Several MEK1/2 inhibitors including selumetinib, MEK162, GSK1120212, CI-1040, PD0325901, and XL518 have either been or are currently being evaluated in phase I/II clinical trials, and still others are in preclinical development (3–5). The survival of many myeloid leukemia cells, both in vitro and in vivo, depends on the activation of this MAPK pathway, and various MEK1/2 inhibitors have been used to successfully inhibit leukemic proliferation (18, 19). In this study we tested four MEK1/2 inhibitors in HL-60 myeloid leukemia cells which harbor an N-RAS mutation and demonstrate constitutive MAPK activation (18, 20).

The most effective preclinical compounds targeting the Raf-MEK1/2-ERK1/2 pathway are against MEK1/2. Because ERK1/2 are the only known substrates of MEK1/2, the proliferative inhibition and reduced survival seen following MEK1/2 inhibition are attributed to ERK1/2-mediated factors (4, 7). PD98059 and U0126 are the most popular preclinical MEK1/2 inhibitors used to study this pathway, and the results obtained with these compounds in cell culture have been used to justify the development of clinical inhibitors. Here we show that these structurally distinct MEK1/2 inhibitors and a newer inhibitor, MEK inhibitor I (MIIC),2 not only block ERK1/2 phosphorylation but also cause acute alterations of mitochondrial electron transport chain (ETC) function.

The ETC is composed of four protein complexes containing electron carriers embedded in the inner mitochondrial membrane and cytochrome c (Cytc) present in the intermembrane space. Complex III, Cytc, and cytochrome oxidase (complex IV) (CytOX) contain heme electron carriers which can either be oxidized (not carrying electrons) or reduced (carrying electrons), and their oxidation state can be measured with multiwavelength cell spectroscopy. Inhibition of the mitochondrial complexes results in an upstream reduction and a downstream oxidation of the electron carriers as well as decreasing oxygen

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consumption allowing multiband spectroscopy, combined with NADH spectroscopy, to pinpoint the site of mitochondrial inhibition in living cells (21).

In this paper, we show that PD98059, U0126, and MIIC potently inhibit the ETC at complex I at concentrations routinely used in the literature for inhibition of MEK1/2, and we investigate the role of this inhibition on human leukemia cell proliferation. Furthermore, we show that PD198306, a newer and more potent MEK1/2 inhibitor, acts as a robust mitochondrial protonophore and uncouples oxidative phosphorylation at higher concentrations. In short, we show the strong mitochondrial effects of these compounds provide a new mechanism by which these inhibitors regulate cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Culture—HL-60 cells were cultured at 37 °C in spinner flasks in phenol red-free RPMI 1640 medium (Invitrogen) containing antibiotic/antimycotic (Sigma) and 10% fetal bovine serum (Invitrogen) in a 95% air and 5% CO2 incubator. Cell viability was determined via trypan blue exclusion using the Countess® Automated Cell Counter (Invitrogen). Proliferation was defined as the increase in viable (trypan blue-excluding) cells over time compared with vehicle control-treated cells. This definition takes into account changes due to cell division, differentiation, and death.

Inhibitors—U0126 (C18H16N2S2) and PD198306 (C18H16F3IN2O2) were purchased from Tocris (Ellisville, MO). PD98059 (C16H13NO3) was from Promega. MEK inhibitor I designated MIIC (C21H18N4OS) was from EMD/Calbiochem. Rotenone and pyridaben were from Sigma. Rotenone was dissolved in ethanol. Pyridaben and the MEK1/2 inhibitors were dissolved in DMSO, and controls were treated with a correlating concentration of DMSO. Inhibitors were added as indicated in the figure legends.

Cell Spectroscopy and Analysis—Cells were spun down at 500 × g for 5 min and then resuspended in at a density of 2.0 × 107 cells/ml in RPMI 1640 medium and placed in a custom-built 5-ml chamber that consisted of a 17-mm inside-diameter quartz crucible embedded in an aluminum block maintained at 37.0 °C by a thermostatic element. The oxygen concentration within the chamber was measured from the fluorescence lifetime of a phosphorescent membrane inserted through a 3-mm-diameter hole in the side of the crucible, and the top of the chamber was sealed with a stainless steel plunger. The stir bar was made of glass rather than Teflon, and all of the seals were made of Viton in accordance with good respirometry practice (22). The cells were oxygenated and deoxygenated under computer control by exchange of oxygen across 80 mm of oxygen-permeable silicone tubing immersed in the cell suspension using a feedback circuit to adjust the oxygen tension within the tubing to maintain constant oxygenation within the chamber; the tubing always contained 5% CO2 to maintain intracellular pH. Oxygen consumption was measured from the difference between the oxygen delivery to the cell suspension by the tubing and the rate change of the oxygen concentration of the cell suspension. The oxygen delivery was calculated from the oxygen gradient across the wall of tubing and the oxygen permeability of the tubing which was measured prior to each study.

Spectroscopy and Spectral Analysis—Heme attenuation spectra and NADH fluorescence spectra were measured with two separate CCD-spectrograph systems working in time-multiplexed mode at 50 Hz using a 6-ms on/4-ms off duty cycle. Contiguous spectra were averaged to give a temporal resolution of 0.5 s. A warm white light emitting diode (LED) was used for the attenuation spectra illumination which was mounted 10 mm below a bundle of three NA0.71-mm optical fibers. One fiber was used for attenuation spectra detection, one for fluorescence spectra detection and one was coupled to a 365-nm UV LED for fluorescence excitation. The two detection fibers were F-matched onto the slits of two 0.3-mm spectrographs (Triax 320; Horiba, Edison, NJ), each equipped with a 1024 × 128-pixel back-thinned CCD camera (DV401BV; Andor Technology, South Windsor, CT). The attenuation spectrograph was equipped with a 600 g/mm grating blazed at 500 nm, which provided complete spectra between 508 and 640 nm with a pixel bandpass of 0.16 nm. The slits were set to give a spectral resolution of 1 nm. The NADH fluorescence spectrograph was equipped with a 300 g/mm grating blazed at 500 nm, which provided complete spectra between 400 and 670 nm with a pixel bandpass of 0.33 nm. The slits were set to give a spectral resolution of 20 nm.

Heme oxidation changes were calculated by fitting a linear combination of model spectra to the change in attenuation spectrum (23) over the wavelength range 520–630 nm. The model spectra were: b4, b1, c1 hemes of complex III, Cyt c, a605, a602, and a5 spectra from Cyt Ox (23) and a quadratic background to account for any base-line drift. All model spectra were measured from the isolated enzymes except a602 (see Ref. 23). The b560 heme of complex II is not included in the model because it has a midpotential of −185 mV (24) and so is unlikely to be reduced in the absence of exogenous reductants. The oxidized fraction of each heme (oxidation state) was calculated from the oxidation changes assuming full reduction during anoxia and full oxidation at high oxygen tension after addition of 1 μM complex I inhibitor rotenone.

NADH oxidation changes were calculated by fitting a linear combination of model spectra to the fluorescence intensity spectrum over the wavelength range 410–610 nm. The model spectra were the fluorescence spectrum of NADH, the fluorescence spectrum of the RPMI 1640 medium and a linear background. The NADH signal originates from both NADH and cytosolic NADH did not change with these interventions. The oxidized state of mitochondrial NADH was calculated from oxidation changes assuming full reduction of mitochondrial NADH during anoxia and full oxidation of mitochondrial NADH after 1 mM complex II inhibitor 3-nitropropionic acid, which inhibits the TCA cycle, and 1 μM protonophore carbonyl cyanide m-chlorophenyl hydradrazine (CCCP), assuming that NADPH and cytosolic NADH did not change with these interventions.

Quantitative Western Blotting—Aliquots containing of 2.0 × 106 cells were removed from the chamber and immediately combined with 500 μl of cold PBS, centrifuged at 4.3 × g for 90 s, and the resulting pellet was resuspended in Laemmli buffer heated to 75 °C and heated for 5 min. The proteins were separated on a 14% SDS-polyacrylamide gel (Novex) and transferred to an Immobilon P membrane. Membranes were blocked in...
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**RESULTS**

**Dose-dependent Inhibition of Proliferation and Phosphorylation of ERK1/2 by Various MEK1/2 Inhibitors**—MEK inhibitors slowed the proliferation of HL-60 cells in a dose-dependent manner that varied with inhibitor (Fig. 1A). Exponentially growing HL-60 cells were treated with 1 nM–300 μM inhibitor for 48 h, and the number of viable cells was calculated and related to the proliferation of vehicle (DMSO)-treated cells. PD98059 showed a slightly stronger inhibition of proliferation per dose than MIIC and U0126. All three of these drugs were more potent at inhibiting proliferation than PD98059, a first generation MEK1/2 inhibitor. A 50% inhibition of proliferation was detected with 300 μM PD98059, the highest concentration tested (Fig. 1A). U0126 and MIIC, at 30 μM, inhibited the proliferation of HL-60 cells by 67 and 77%, respectively. Phosphorylation of ERK1/2, the only known substrates of MEK1/2, is the standard assay of MEK1/2 activity. To determine the MEK1/2 activity of inhibitor-treated HL-60 cells, total cell extracts were made 2 min after exposure to 1–100 μM inhibitor. Western analysis indicated that little or no pERK1/2 was found following 1 μM MIIC, U0126, and PD98059 and 100 μM PD98059 (Fig. 1B).

**Direct Effects of MEK1/2 Inhibitors on the ETC and Evidence for Complex I Inhibition**—Multiwavelength cell spectroscopy was used to quantitate oxidation changes of the ETC hemes simultaneously with oxygen consumption (VO2). A typical study is shown in Fig. 2 where a downward deflection in Cytc denotes reduction and an upward deflection denotes oxidation. Oxygen concentration within the chamber was maintained at 100 μM (~10% O2) except in the gray shaded area where the O2 concentration was altered under computer control. O2 was decreased to zero (anoxia) and maintained there for 2 min then returned to 100 μM. Under anoxic conditions, electrons are not able to leave the chain, and all of the upstream redox centers, including Cytc, become fully reduced (Fig. 2, A and B). The addition of 1 μM rotenone, a complex I inhibitor, blocked entry of electrons into the chain, resulting in a complete oxidation of Cytc and blocked all mitochondrial VO2 (Fig. 2, A and B). The heme oxidation states were subsequently calculated assuming that the hemes were fully reduced and fully oxidized under anoxia and rotenone, respectively. The addition of U0126 indicated by the arrows, to final concentrations of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM, resulted in a concentration-dependent decrease in VO2 and oxidation of Cytc (Fig. 2, B and D).

At base line 62 ± 1% of Cytc, 69 ± 4% of b1 of the bc1 complex (complex III) and 89 ± 3% of CytOx were oxidized (Fig. 3, top). DMSO, given as a vehicle control, slightly increased VO2 but did not appreciably change the cytochrome oxidation state (Fig. 3, left). The addition of MIIC and U0126 caused a dose-dependent oxidation of Cytc, b1, and CytOx (Fig. 3, top). Cytc was 97.5 ± 0.3% and 88.5 ± 0.2% oxidized after 10 μM MIIC and U0126, respectively, and nearly fully oxidized after 30 μM. The b1 and c1 hemes of the bc1 complex also oxidized in concert with the other hemes (data not shown). The oxidation was rapid and stabilized within 90 s (Fig. 2D). PD98059 also oxidized the hemes but required more drug, 30 μM and higher.
FIGURE 2. U0126 acutely inhibits VO₂ and Cytc oxidation in a concentration-dependent manner. Representative data were obtained from the multiwavelength cell spectroscopy system. Gray denotes areas where [O₂] is < 100 μM, and arrows show where inhibitor, either U0126 or 1 μM rotenone (R), was added. Oxygen consumption (VO₂) is shown in A and B. Change in Cytc oxidation from base line is shown in C and D. A and C show a simple anoxia and rotenone study to fully reduce and fully oxidize the hemes. B and D show an anoxia, addition of U0126 to a final concentration of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM, and finally rotenone.

FIGURE 3. Concentration-dependent inhibition of ETC by structurally distinct MEK1/2 inhibitors. Oxidation state of heme b₅₅ of complex III, Cytc, and heme a of CytOx (upper panels), and oxygen consumption as a percentage of base line (lower panels) 2 min after treatment with 1 nM–30 μM MIIC, U0126, and PD198306, 1–100 μM PD98059, or equivalent amounts of vehicle-control (DMSO). Results are expressed as mean ± S.D. (error bars; n = 6). Cytc changes were significant (p < 0.005) at concentrations of 0.03 μM and higher for MIIC and U0126 (p < 0.05) at and above 0.01 μM PD198306 and 3 μM PD98059. VO₂, CytOx, and Cytb₅₅ changes were significant (p < 0.05), respectively, at the following concentrations: MIIC 0.3, 0.3, 1; U0126 0.1, 3, 10; PD98059 10, 100, 30; PD198306 0.03, 10, 10. Direct linear correlation between Cytc oxidation and VO₂ was found for MIIC, U0126, and PD98059. Cytc oxidation and VO₂ were measured 2 min after treatment with varying doses of inhibitor. The linear regression for each inhibitor was R² > 0.99. Changes in Cytc oxidation state (upper panels) and NAD(H) oxidation state (lower panels) were measured following treatment with 0.001–10 μM MIIC, U0126, or PD198306. Measurements were taken 2 min after each dose. Results are expressed as mean ± S.D. (n = 3). p < 0.01 at concentrations of 1 μM U0126, 0.1 μM MIIC, and 10 μM PD198306 and higher.
to do so. PD198306, a newer generation MEK1/2 inhibitor, had little effect on the heme oxidation state until the highest concentration, 30 μM. A dose-dependent decrease in VO₂ was noted following treatment with MIIC or U0126 (Fig. 3, bottom). Similarly, PD98059 decreased VO₂ but required a greater concentration of drug. In contrast, PD198306 first increased VO₂ at moderate concentrations (3 and 10 μM) and then decreased VO₂ at the highest concentration (30 μM), where it also oxidized Cytc and b₅₆₅. U0124, sold as a small molecule control for U0126 studies, had no effect on VO₂ or cytochrome oxidation state (data not shown).

A direct linear correlation, R²>0.99, between Cytc oxidation and VO₂ was found for MIIC, U0126, and PD98059 (Fig. 3, lower left). As each of these compounds was added Cytc oxidation increased in concert with a decrease in VO₂. The results suggest that these inhibitors have the same mechanism of action on the mitochondria.

The oxidation of all ETC hemes along with inhibition of VO₂ indicated that the MEK1/2 inhibitors were disrupting the ETC upstream of complex III. Similar to the hemes, NAD⁺ and NADH are a redox couple where NADH is the reduced form (carrying two electrons) and NAD⁺ is the oxidized form. To determine whether the inhibition was occurring at complex I or upstream of complex I, the oxidation state of NAD(H) was measured fluorometrically. Treatment with MIIC and U0126 resulted in a concentration-dependent reduction in NAD(H) which was significant starting at 0.1 μM and 1 μM (p < 0.005), respectively (Fig. 3, lower right). The effect of PD98059 could not be determined because the drug itself fluoresced. U0126 also fluoresced, but it could be spectrally resolved. PD198306 resulted in an oxidation in NAD(H) at 10 μM.

Complex I and Proliferative Inhibition Independent of MEK1/2 Inhibition—The concentration of each inhibitor required to reduce phosphorylation of ERK1/2 to 5% of baseline produced only a 10–25% decrease in proliferation compared with vehicle-treated control cells at 48 h (Fig. 4A, gray section). Improved proliferative inhibition to 80% of control occurred at higher doses, with little or no further decrease in pERK1/2 levels. For the inhibitors MIIC, U0126, and PD98059 this improved proliferative inhibition strongly correlated with the oxidation of Cytc and a decrease in VO₂ (Fig. 4, B and C). These changes are indicative of inhibition of the ETC. Similarly, PD198306 inhibits proliferation by just 25% when pERK1/2 is <5% of control. A greater proliferative inhibition was noted at higher doses, which did not decrease pERK1/2 levels further but did alter mitochondrial function.

Rotenone, an established complex I inhibitor, caused no decrease in pERK1/2/ERK1/2 although there was significant inhibition of proliferation at 48 h to 18 ± 6% of vehicle-treated control cells, suggesting loss of pERK1/2 is not necessary for complex I inhibition (Fig. 4D and F). As expected, 1 μM rotenone fully oxidized Cytc and inhibited VO₂ to 6.2 ± 2.4% of control; the residual VO₂ was nonmitochondrial in origin (Fig. 4E). Another known complex I inhibitor, pyridaben (1 μM), also oxidized Cytc, abolished mitochondrial VO₂, and inhibited...
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proliferation to 44 ± 4% of control (data not shown), showing that complex I inhibition robustly affects proliferation.

At 1 μM, MIIC and U0126 had only modest effects on proliferation despite lowering pERK1/2/ERK1/2 to <2.5% of control levels. This concentration caused partial inhibition of complex I as indicated by the intermediate inhibition of VO₂ and oxidation of ETC cytochromes (Figs. 4E and 3). At a higher concentration of 10 μM, MIIC and U0126 further decreased VO₂ and further inhibited proliferation but caused no detectable change in VO₂ in isolated HL-60 mito- 

cyclochondria (data not shown), suggesting that U0126 acts to 

mitochondria whose physiological substrate is complex I. The mammalian complex I contains 45 subunits (30), and some of the subunits reportedly contain phosphorylation sites (31, 32) although consensus has not been reached as to their function- 

ality and relevance (33).

Low concentrations of a protonophore decrease the mem- 

brane potential and stimulate respiration whereas high concen- 

trations cause a decrease in respiration concomitant with a fail- 

ure in the TCA cycle to reduce NADH (34). Our previous work 

with the protonophore CCCP has shown the Cyt oxidation 

state changes very little at low concentrations, where VO₂ 

increases, but becomes highly oxidized at higher concentra- 

tions where VO₂ decreases (35). PD198306 produced exactly 

this pattern of oxidation and electron flux changes consistent 

with it acting as a protonophore. Increases in VO₂ can also arise 

if ATP consumption is increased, but the changes observed 

with PD198306 also occurred in the presence of the ATP syn- 

thase inhibitor oligomycin, indicating that ATP consumption is 

not driving the increased VO₂ (data not shown). Similar to 

CCCP and another protonophore, carbonyl cyanide p-triflu- 

oromethoxyphenyldrazone (FCCP), PD198306 contains a sec- 

ondary amine group which, in this case, bridges two benzene 

rings. Loss of the proton on the amine group would lead to a 

negative charge that would be delocalized over the two benzene 

rings, thus explaining the protonophore activity at higher con- 

centrations. It should be noted that PD198306 is a structural 

analog of the MEK1/2 inhibitors, CI-1040 and MEK162, used in 

clinical trials.

In summary, the role of the MAPK signaling pathway in 

cellular proliferation is well established, and the overexpression or activation of the upstream components of this pathway in cer- 

tain cancers is undeniable; however, the use of MEK1/2-specific inhibitors to define the function of this pathway must be accepted 

with caution. As we have clearly shown, MEK1/2 inhibitors of diverse structure can alter mitochondrial metabo- 

lism in addition to depleting pERK1/2. Because the reduction of 

pERK1/2 to <5% of base line corresponded to only a 10–25% 

decrease in proliferation compared with control regardless of 

the inhibitor used and because the mitochondrial inhibition 

continued to correlate with enhanced proliferative inhibition, 

we propose a prominent role for mitochondrial-mediated pro-

DISCUSSION

Using a new technology that provides a real-time depiction of 

mitochondrial metabolism in intact cells via measurements of 

NAD(H) oxidation state, heme oxidation states, and oxygen 

consumption, we found that several structurally distinct 

MEK1/2 inhibitors acutely and effectively altered mitochondrial metabolism. Three of the inhibitors tested, U0126, MIIC, and PD98059, had dose-dependent immediate effects on complex I of the ETC. Another MEK1/2 inhibitor, PD198306, acted acutely as a protonophore. As expected, all of the MEK1/2 inhibitors blocked phosphorylation of ERK1/2 in a dose-depen- 

dent manner, and all slowed the proliferation of HL-60 human 

leukemia cells. Notably, the depletion of pERK1/2 to levels 

down to 80% of control, occurred at higher doses of inhibitor 

and correlated strongly with the effects on the mitochondrial 

electron transport.

The immediate, dose-dependent reduction in NAD(H) and 

oxidation in heme b₅₆ of complex III, Cytc, and heme a of 

CytOx, as well as the corresponding decrease in VO₂ caused by 

MIIC, U0126, and PD98059 are hallmarks of complex I inhibi- 

tion. Such inhibition would explain the increase in the ADP: 

Mitochondria (data not shown), suggesting that U0126 acts to 

inhibit complex I independently of cytoplasmic MEK1/2. At 

least 60 different families of compounds have been found to 

inhibit complex I, highlighting its sensitivity to a vast variety of 

structurally unrelated natural and artificial compounds (26).

The oxidation of Cytc in HL-60 cells was maintained for at least 2 days following a single dose of U0126 (data not shown), sug- 

gesting that the inhibition is prolonged.

It is possible that the structurally distinct inhibitors U0126, 

MIIC, and PD98059 bind directly to complex I. Alternatively, 

inhibition could be occurring through a signaling event. Such a 

signaling cascade would require a terminal signaling protein 

with access to complex I and therefore present in either the 

mitochondrial intermembrane space or the mitochondrial 

matrix. There have been several reports of the presence of 

ERK1/2 in mitochondria, but it is difficult to reconcile the mito- 

ochondrial location of ERK1/2 with the impermeability of the 

outer mitochondrial membrane to proteins lacking a mito-

ochondrial import sequence (27–29). In any case, inhibition of 

complex I by MEK1/2 or ERK1/2 can be ruled out because low 

dose PD198306 resulted in the profound loss of pERK1/2, 

presumably indicating complete inhibition of MEK1/2 kinase 

activity, with no observable inhibition of complex I. Kinase 

inhibitors are notoriously nonspecific, and it remains possible 

that these compounds are targeting a kinase within the mito-

ochondria whose physiological substrate is complex I. The mam-

malian complex I contains 45 subunits (30), and some of the 

subunits reportedly contain phosphorylation sites (31, 32) 

although consensus has not been reached as to their function- 

ality and relevance (33).
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The concentrations used to obtain this level of proliferative inhibition were consistent with those published in the majority of literature related to these MEK1/2 inhibitors (36–41). Therefore, it is likely that many of the antiproliferative effects associated with these inhibitors are due to inhibition of mitochondrial function rather than strictly being mediated by the loss of pERK1/2.

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