SU1498, an Inhibitor of Vascular Endothelial Growth Factor Receptor 2, Causes Accumulation of Phosphorylated ERK Kinases and Inhibits Their Activity in Vivo and in Vitro

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SU1498, an inhibitor of vascular endothelial growth factor receptor 2, has been used successfully to study the physiological manifestations of receptor functions. Here we report that in addition to its anti-receptor activity, SU1498 stimulates accumulation of phosphorylated ERKs in human umbilical vein endothelial cells and in human aortic endothelial cells in a manner that is dependent on the functioning of the upstream components of the MAPK pathway, B-Raf, and MEK kinases. The enhanced accumulation of phospho-ERKs is observed only in cells that have been stimulated with sphingosine 1-phosphate or protein growth factors; SU1498 by itself is ineffective. We show that the inhibitor acts by blocking the kinase activity of phospho-ERK both in a direct assay and in immunoprecipitates from cells treated with the compound. The data reveal a novel and unique way in which MAPK signaling pathway may be blocked in human endothelial cells.

Endothelial cells are an integral component of vasculature, and their ability to sense extracellular stimuli is an essential feature of the angiogenic response (1). To the extent that this response can be mimicked in vitro, it consists of cell migration (chemotaxis) and morphological differentiation leading to assembly of a network of tubes (resembling capillaries); these phenomena are induced by factors such as VEGF or a bioactive phospholipid sphingosine 1-phosphate (SPP). The cells possess specific receptors that bind and process these factors: the tyrosine kinase receptors for VEGF (Flt1 or VEGF receptor 1 and KDR or VEGF receptor 2), and the members of the endothelial differentiation gene family of G protein-coupled receptors for SPP, in particular EDG-1 or S1P1 (2, 3). Interestingly, SPP-treated cells exhibit an enhanced level of KDR phosphorylation (4), indicating that the two types of receptors communicate with each other in addition to signaling through the pathways specific to themselves. Similarly, in vascular smooth muscle cells, SPP promotes PDGF receptor phosphorylation (5), and EDG-1 receptor appears to play a role in PDGF-induced migration in these cells (6), consistent with the documented physical tethering of the two receptors (7).

Receptor activation has profound consequences on cell fate, resulting as it does in activation of diverse pathways both proliferative and those leading to differentiation or apoptosis (8, 9). These pathways include the MAP kinase cascades, the phosphatidylinositol 3-kinase-dependent cascades, and receptor-protein-tyrosine kinase cascades, with significant overlaps between them (10–12). Many of these pathways have been fruitfully dissected with the help of inhibitors. Thus, G protein-coupled receptors are often sensitive to pertussis toxin or cholera toxin (13, 14), MAP kinase pathways can be blocked with PD98059 (15, 16), phosphatidylinositol 3-kinase activity is inhibited by the fungal metabolite wortmannin (17), and receptor-protein-tyrosine kinases are sensitive to a large number of compounds known as tyrphostins (18, 19) as well as to other inhibitors.

We have been interested in the biochemical mechanisms influencing the ability of human umbilical vein endothelial cells (HUVECs) to migrate and form capillaries in the presence of VEGF and other stimuli. We observed that compounds purported to be specific blockers of the VEGF receptor, KDR, substantially inhibited responses to SPP as well as to HGF and basic FGF (bFGF). This suggested that these factors induce migration and tube formation through a common pathway or intermediate(s). Because the role of phosphatidylinositol 3-kinase and MAP kinase pathways in migratory reactivity of a variety of cells is well documented (20–28) we have examined the effect of several receptor inhibitors on the state of Akt and ERK phosphorylation. Our results demonstrate that the potent KDR inhibitor SU1498 (29, 30) accentuates accumulation of phosphorylated ERK in endothelial cells and inhibits its kinase activity while having no effect on phosphorylation of Akt.

EXPERIMENTAL PROCEDURES

Cell Cultures—HUVECs were obtained from VEC Technologies, Inc. (Rensselaer, NY) and grown to no more than passage 10 in RPMI 1640 or DMEM-based medium containing endothelial cell growth supplement (30 μg/ml; Upstate Biotechnology, Lake Placid, NY), heparin sulfate (2 units/ml), 25 mM glucose, 20 mM l-glutamine, antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml; amphotericin B, 0.25 μg/ml), and 20% fetal bovine serum. When necessary cells were starved in the same medium containing 0.5% serum. Chemotaxis and tube formation assays were carried out as described previously (31). Human aortic endothelial cells were obtained from Cambrex Bio Science (Walk-
ersville, MD) and were grown in appropriate cell media (bullet kits) provided with the cells. Human aortic smooth muscle cells were also obtained from Cambrex Bio Science and were grown in a medium containing DMEM, 25 mM glucose, 20 mM l-glutamine, antibiotics, and 10% fetal bovine serum.

**Materials**—Media components were from Invitrogen and from Cambrex Bio Science. Culture flasks and plates and Transwell chemotactic chambers and filters were purchased from Fisher. Precast polyacrylamide gels, electrophoresis buffers, and nitrocellulose membranes were purchased from Invitrogen, and Western blocking reagent was from Roche Applied Science. Media components were from Cambrex Bio Science and grown in a medium containing DMEM, 25 mM glucose, 20 mM l-glutamine, antibiotics, and 10% fetal bovine serum.

**Chemicals and Antibodies**—Sphingosine 1-phosphate was from Sigma and the various inhibitors used in this study, including SU1498, AG1433, AG1296, were obtained from Calbiochem. Growth factor-reduced Matrigel was obtained from Collaborative Biomedical Products (Bedford, MA). Protein growth factors, VEGF, PDGF-BB, EGF, HGF, bFGF were from R&D Systems (Minneapolis, MN). Rabbit polyclonal anti-phospho-Akt (Ser-473), anti-ERK1/2, and anti-phospho-ERK1/2 antibodies were from Cell Signaling (Beverly, MA) as was the p44/42 MAP kinase assay kit that included activated murine ERK2. Human activated ERK1 was purchased from Calbiochem. Mouse monoclonal anti-actin antibody was from Santa Cruz Biotechnology Corp. (Santa Cruz, CA).

**Preparation of Cell Lysates, SDS-PAGE, and Western Blotting**—Cells grown in plates or flasks were serum-starved as indicated and rinsed with PBS. After the treatments, the cells were lysed with the lysis buffer (50 mM Tris-HCl, pH 7.2–7.4, 1% Triton X-100, 0.5 M NaCl, 0.125% sodium deoxycholate, 0.1% SDS, 10 mM MgCl2) containing 2 mM NaF, 0.2 mM sodium pervanadate, aprotinin (20 μg/ml), leupeptin (20 μg/ml), and 1 mM phenylmethylsulfonyl fluoride. Lysates were mixed with one-fourth the volume of the 4× electrophoresis sample buffer (Invitrogen) containing 1% mercaptoethanol, heated 5 min at 99°C, and stored at −80°C until use. Samples of the lysates were fractionated by SDS-PAGE, proteins were transferred electrophoretically to nitrocellulose membranes, blocked with 10% Western blocking reagent from Roche Applied Science.

**Immunofluorescence Staining**—HUVECs (20,000–100,000 cells) were grown in appropriate cell media (bullet kits) provided with the cells. Human aortic smooth muscle cells were also obtained from Cambrex Bio Science and were grown in a medium containing DMEM, 25 mM glucose, 20 mM l-glutamine, antibiotics, and 10% fetal bovine serum.
Addition of 40 mixtures were incubated for 10 min at 25 °C.

Cells were inoculated into the wells of 6-well culture plates (3 x 10^4 cells/well) and grown for 16 h in DMEM-based medium at 37 °C in the atmosphere of 5% CO₂. After an overnight starvation (0.5% fetal bovine serum), the medium was removed, and the cells were washed with PBS and overlaid with 0.4 ml of DMEM. From this point on, all incubations were done at 25 °C. The inhibitors (SU1498, 10 μM; AG1433, 10 μM; AG1296, 25 μM; PD153035, 1 μM) were added and followed 15 min later by the growth factors: SPP, 1 μM; VEGF, 100 ng/ml; HGF, 100 ng/ml; bFGF, 10 ng/ml; EGF, 10 ng/ml. Forskolin (10 μM), a gratuitous inducer of adenyl cyclase, was used as a control because of its known ability to enhance ERK phosphorylation (66). After a 10-min incubation, the medium was siphoned off, and the cells were washed with PBS and overlaid with 0.8 ml of DMEM. The cells were then starved overnight, the medium was aspirated, and the remaining chamber was filled with 1 ml of each compound and chambers were held at room temperature for the duration of treatment (see Fig. 6). Cells were then washed with PBS, fixed for 10 min with 2 ml of cold 3% paraformaldehyde, and washed 3 times with PBS. The fixed cells were permeabilized in 0.1% Nonidet P-40 in PBS and secured with coverslips sealed with Gel/Mount (Biomeda Corp., Foster City, CA). Images were obtained with the aid of an epi-illuminated inverted microscope (Diagnostic Instruments, Sterling Heights, MI).

**ERK Activity Assays**—A kit from Cell Signaling was used for this purpose. This kit included the kinase buffer, ATP, and an active form of ERK2 kinase and its protein substrate, Elk1, whose phosphorylation was detected with anti-phospho-Elk1 antibody. In the direct assay the active ERK1 or ERK2 was diluted to 1 ng/μl with the kinase buffer without ATP. Separately, the Elk1 protein was diluted to 20 μg/ml with the kinase buffer containing 50 μM ATP. The ERK1 or ERK2 solution was pipetted into tubes (1 μl per tube) and mixed with 0–10 μl of 50 μM SU1498 (in kinase buffer without ATP). The blank tube received buffer only. The volume was adjusted to 11 μl with the same buffer, and the mixtures were incubated for 10 min at 25 °C. This was followed by the addition of 40 μl of the Elk1-ATP buffer solution, and the incubations were continued for 30 min at 30 °C. The reactions were stopped with 20 μl of 4 × sample buffer mix and heating at 95 °C for 10 min. Samples (15 μl) were fractionated by SDS-PAGE, and phosphorylated Elk1 was detected by immunoblotting with anti-phospho-Elk1 antibody.

The immunoprecipitation assay was performed essentially as instructed by the manufacturer.HUVECs were grown in T150 culture flasks (2 x 10⁶ cells/flask) and starved overnight. The medium was siphoned off, and the cells were washed with PBS and overlaid with 4 ml of DMEM. SU1498 was added to selected flasks for 15 min followed by SPP (1 μM) or VEGF (100 ng/ml). A control flask was left untreated. After a 10-min incubation the cells were lysed with 0.8 ml of lysis buffer provided with the kit, and clear supernatants were harvested by centrifugation for 10 min at 15,000 × g at 4 °C. Each supernatant received a 15-μl aliquot of immobilized anti-P-ERK antibody, and the suspensions were incubated with gentle agitation overnight at 4 °C. The precipitates were collected by centrifugation, and the pellets were suspended in 50 μl of the kinase buffer containing 200 μM ATP and 2 μg of Elk1 protein. The mixtures were incubated 30 min at 30 °C, and the reactions were stopped with 20 μl of 4 × sample buffer. Samples (15 μl) were fractionated by SDS-PAGE, and phosphorylated Elk1 was detected by immunoblotting with anti-phospho-Elk1 antibody.

**RESULTS**

Tyrosine Kinase Receptor Inhibitors Interfere with HUVEC Migration and Tube Formation in Response to SPP or VEGF—Receptor-mediated chemotactic and morphogenetic responses to bioactive lipids and protein growth factors are essential to the angiogenic process. With this in mind we examined the effect of several receptor inhibitors on cell migration and capillary network formation. Each inhibitor was able to block chemotaxis to both SPP and VEGF (Fig. 1A). Furthermore, the same inhibitors diminished assembly of tube networks that the cells produced in response to SPP and several angiogenic growth factors (Fig. 1B). Interestingly, despite the reported absence of PDGFR in endothelial cells (32–34), the PDGF receptor inhibitor AG1296 inhibited migration profoundly, albeit at a high concentration; it also affected tube formation induced by factors unrelated to PDGF.

SU1498-treated Endothelial Cells Accumulate Phosphorylated ERK1/2—The above results suggested that the inhibitors that we used might exert their effect not only at the level of receptors but also at some point(s) downstream in the signaling pathway(s). We considered the possibility that the inhibitors might act to prevent phosphorylation of MAP kinases or Akt/protein kinase B, thereby inactivating a major signaling component of the cell. Surprisingly, this was not the case. Even though the levels of phosphorylated Akt remained low regardless of treatment, there was a dramatic upward shift in phosphorylated MAP kinases at 15 min and increasing at higher concentrations (Fig. 3A, 3B, and 3C). The phosphorylation of ERK1/2 at Thr180/Tyr182 was stimulated by factors unrelated to PDGF.

Migration experiments demonstrated that the SU1498-dependent buildup of phospho-ERK required the presence of SPP or VEGF (Figs. 3 and 4). The accumulation was dependent on the length of exposure to these stimuli (Fig. 3A), but prolonged incubation with the inhibitor resulted in cell death and decrease of recoverable phospho-ERK (Fig. 3B). We have also determined that the extent of ERK phosphorylation correlated with the concentration of SU1498, reaching a maximum at the 10 μM concentration of the compound and decreasing at higher concentrations (Fig. 3C).

G. Boguslawski, unpublished data.
SU1498-induced ERK Phosphorylation Requires the Activity of Signaling Pathways—To gain insight into the manner in which SU1498 exerts its influence, we examined the consequences of blocking the activity of MEK, p38 kinase, PKA, and PKC on the accumulation of phospho-ERK1/2. As demonstrated in Fig. 4A, the various compounds, used individually, had no appreciable effect on the level of detectable phospho-ERK1/2, forskolin being the only exception. Again, even though neither SPP nor VEGF by themselves elicited a strong P-ERK1/2 response, the presence of these stimuli was essential for SU1498-mediated accumulation (Fig. 4B). This accumulation as well as that stimulated by forskolin was completely abolished when the MEK inhibitor, PD98059, was present; thus, ERK phosphorylation proceeds through the well-established MAP kinase signaling pathway. Also intriguing was the fact that PD169316, a specific inhibitor of p38 kinase, interfered strongly with ERK phosphorylation stimulated by SU1498. Although the manner in which p38 kinase may participate in ERK activation in HUVECs is not well understood, the observed effect is consistent with cross-talk between the ERK and p38 kinase pathways (35). We note that okadaic acid, an inhibitor of phosphatase PP2A, was without effect (Fig. 4C).

Neither 2 μM SU6656, an Src family kinase inhibitor (36), nor 20 μM genistein, a less-specific protein-tyrosine kinase inhibitor, had any effect on SU1498 action; however, PKC inhibitors bisindolylmaleimide I, a broad spectrum PKC inhibitor (1 μM) and Go6983 (5 μM) abolished SU1498-stimulated ERK phosphorylation (Fig. 4). Therefore, the enhanced phosphorylation of ERK does not appear to be dependent on tyrosine kinases but requires protein kinase C.

We extended these observations to two additional cell lines, human aortic endothelial cells and human aortic smooth muscle cells. As shown in Fig. 4D, the response of human aortic endothelial cells to treatment with SU1498 was exactly the same as that seen with HUVECs, the inhibitor potentiating the accumulation of phospho-ERKs in cells stimulated with SPP or HGF. However, smooth muscle cells did not exhibit an increase in phosphorylated ERKs, perhaps because of the high basal level of P-ERK1/2 in these cells (Fig. 4E) or because of a difference in the manner human aortic smooth muscle cells regulate ERK activity (see “Discussion”).

The observation that forskolin, an inducer of adenylyl cyclase, stimulated ERK phosphorylation (Figs. 2 and 4) implied the involvement of cAMP-dependent protein kinase (PKA) in the activation of MAPK pathway in HUVECs. We tested this further with the help of H89, a commonly used inhibitor of PKA (37). As is apparent from Figs. 4D and 5, the addition of H89 caused a substantial decrease in the extent of ERK phosphorylation. Furthermore, we have noted that a Raf-1 kinase inhibitor not only did not inhibit but actually enhanced phospho-ERK accumulation, an effect strongly counteracted by H89. These results coupled with insensitivity to wortmannin and to Src tyrosine kinase inhibitor indicate that HUVECs utilize the PKA-dependent B-Raf kinase in preference to Raf-1 kinase to...
phosphorylate MEK and ERK (38–40).

**SU1498 Has No Effect on Nuclear Localization of ERK1/2**—Phosphorylation of ERKs results in the movement of a fraction of the kinase pool to the nucleus, where the kinases activate a number of transcription factors including c-Fos, c-Jun, and Elk1 (41–43). We used anti-phospho-ERK antibody binding...
followed by decoration of the complex with fluorescein isothiocyanate-conjugated secondary antibody to examine the effect of SU1498 on nuclear translocation of phosphorylated ERKs in HUVECs (Fig. 6). Cells were manipulated as described under “Experimental Procedures” and analyzed for fluorescence. We noted that even the unstimulated cells showed a significant amount of phosphorylated ERKs in the nuclei. It is not known whether this indicates a high basal activity of the MAPK pathway in HUVECs. Consistent with the weak stimulation of ERKs by SPP and VEGF, the nuclei of cells treated with these agents alone showed only slightly enhanced staining with anti-phospho-ERK antibody. We did not observe much difference when SU1498 was present except that some of the fluorescent nuclei appeared grainier, with a punctate pattern readily apparent. Formation of phospho-ERK aggregates has been reported in neuronal cells treated with 6-hydroxydopamine (44), but we do not know if the grains we observe are analogous to those aggregates. We conclude that SU1498 does not substantially affect nuclear translocation of phospho-ERKs.

**SU1498 Inhibits ERK Activity—**Functionality of MAPKs is generally controlled by their phosphorylation state (11), and a number of phosphatases are known to specifically interact with phospho-ERKs and cause their dephosphorylation (45–49). Therefore, it is possible that SU1498 inhibits a phosphatase, thereby preventing ERK dephosphorylation and leading to accumulation of phospho-ERK. Alternatively, and not necessarily in contradiction, SU1498 may inhibit phospho-ERK directly, inactivating it and also preventing it from being accessed by the phosphatase. We used a phospho-ERK immunoprecipitation kit and Elk1 protein substrate to determine the effect of SU1498 on ERK activity. In a direct enzymatic assay we found that the purified preparations of active human ERK1 or murine ERK2 were strongly inhibited by SU1498; as the concentration of the inhibitor increased, the amount of phospho-Elk1 decreased to a barely detectable level (Fig. 7A). Consistent with these results, phospho-ERK immunoprecipitated from lysates of SU1498-treated HUVECs was significantly impaired in its ability to phosphorylate Elk1 protein (Fig. 7B).

**DISCUSSION**

The application of inhibitors to the study of biological processes is a well entrenched and successful approach. However, there are two major risks inherent in such an approach, that of lack of specificity and that of side effects. This may be particularly true of protein kinase inhibitors by virtue of the fact that these enzymes are broadly distributed and broadly involved in countless metabolic reactions. A careful study by Davies et al. (50) demonstrated that many of the most commonly used kinase inhibitors could be quite promiscuous in their choice of targets. Frequently, the presumed protein kinase inhibitors affect enzymes completely unrelated to kinases (51, 52). This does not necessarily mean that such molecules are worthless; in fact, the additional targets of their action may make them more valuable.

In this communication we have examined the effect of several tyrosine kinase receptor inhibitors on the ability of HUVECs to migrate and to form a network of capillaries in response to angiogenic stimuli. Our data demonstrate that at least three separate inhibitors are able to diminish migration of cells toward SPP or VEGF. The same inhibitors also decrease the extent of tubuloneogenesis in cells exposed to these and other growth factors (Fig. 1). Thus, SU1498, AG1433, and AG1296 have an inhibitory effect on the responses induced not only by SPP and VEGF but also by HGF and bFGF, protein factors whose receptors are not known to be blocked by these compounds. Strikingly, even though mature endothelial cells are generally devoid of PDGF receptors, they are sensitive to the action of AG1296, a potent inhibitor of PDGFRA (32–34). However, there are suggestions that neovascular, capillary,
and microvascular endothelial cells from several sources do express an active PDGFR (53-55).

The relationship between VEGF and its receptor KDR and other growth factors is a complex one. It has been demonstrated that bFGF induces expression of KDR in bovine retinal endothelial cells (12). PDGF can promote VEGF expression in porcine aorta endothelial cells (54), and HGF was shown to increase expression of VEGF in human keratinocytes and of KDR in endothelial cells (57). Thus, it is conceivable that agents that inhibit KDR will also prevent bFGF or PDGF from promoting chemotaxis and/or capillary formation because they prevent expression of VEGF or VEGF receptors induced by bFGF or PDGF. It is also possible that some of the inhibitors share a common target not directly related to the cognate receptor itself.

We discovered that SU1498, a powerful inhibitor of KDR (IC_{50} = 0.7 \mu M), stimulated accumulation of phosphorylated ERK1/2 in endothelial cells (Fig. 2), although not in smooth muscle cells, indicating that the MAPK pathway is at least one common thread in the responses induced by any of the growth factors we tested. As shown in Figs. 3 and 4, the accumulation required the presence of the stimulus, SPP or VEGF, and was both time- and dose-dependent. Furthermore, the process was stimulated by forskolin (Figs. 2 and 4), depended on the activity of MEK, and was blocked by H89, a protein kinase A inhibitor, but not by Raf-1 kinase inhibitor (Fig. 5) or by the Src kinase family inhibitor, SU6656 (Fig. 4). The Raf family of kinases consists of three isoforms, Raf-1 (C-Raf), B-Raf, and A-Raf (40, 58), of which Raf-1 and A-Raf are broadly distributed, whereas B-Raf has a more restricted pattern of expression (59). The B-Raf isoform is activated by PKA (38, 39) and is, therefore, sensitive to H89. Because Raf-1 kinase is not sensitive to H89 and because the Raf-1 kinase inhibitor did not block SU1498-stimulated accumulation of phospho-ERK1/2 whereas H89 did, we conclude that HUVECs utilize B-Raf kinase to activate MAPK pathway. To our knowledge this is the first indication that, under our experimental conditions, endothelial cells employ B-Raf kinase to activate MAPK pathway (60).

In our hands induction of ERK phosphorylation by SPP or VEGF was weak, perhaps because HUVECs possess highly active dephosphorylation machinery, but it was strikingly enhanced in the presence of SU1498 in time-dependent fashion. Although the exact mechanism of SU1498 action is not known, our data suggest that the inhibitor may act as a molecular trap for phosphorylated ERK proteins. In this view (Fig. 8), under normal conditions ERKs are rapidly phosphorylated and then dephosphorylated by any of the number of phosphatases (45, 47, 48). The presence of SU1498 would prevent dephosphorylation and resulting in accumulation of the phosphorylated protein. This view is supported by our data showing the decrease in phosphorylation of Elk-1 by ERK1/2 both in vitro and by phospho-ERKs immunoprecipitated from cells treated with the inhibitor (Fig. 7). However, SU1498 was ineffective in smooth muscle cells, suggesting that these cells may utilize a different, inhibitor-insensitive phosphatase to dephosphorylate ERKs (Fig. 4E). The existence of cell type-specific phospho-ERK phosphatases has been well documented (45-49, 61, 62). Because ERKs of both murine and human origin are equally sensitive to SU1498 (Fig. 7), it is likely that the lack of SU1498 effect on P-ERK1/2 accumulation in human aortic smooth muscle cells is caused not by a peculiarity of the ERKs themselves but, rather, is the result of distinct regulatory mechanisms at work in this cell type.

SU1498 is not the only inhibitor known to enhance the accumulation of phospho-ERK. As noted earlier, AG1296, a powerful inhibitor of PDGF receptor, was also able to promote the buildup of phospho-ERK in HUVECs. Whether this inhibitor acts in the same manner as SU1498 has not yet been determined. Interestingly, Taxol, an antitumor agent that exerts its antineoplastic effects by stabilizing microtubules and causing mitotic arrest and apoptosis, has been reported to increase the
level of phosphorylated ERKs in MCF7 breast cancer cells (63, 64). However, in contrast to SU1498, the effect of taxol was manifest only upon prolonged incubation, and there was no evidence of inhibition of kinase activity.

Our data demonstrate again that caution is necessary in interpreting the results of studies involving the use of metabolic inhibitors (50–52) and indicate that mere accumulation of phospho-ERKs cannot be assumed to reflect MAPK pathway functioning unless the enzymatic activity of the accumulated protein is determined. Excitingly, the results also reveal the heretofore unsuspected and, to our knowledge unique ability of a known KDR blocker selectively to inhibit ERK in its phosphorylated state. In this, SU1498 is unlike the known MEK inhibitor, PD98059, which acts by preventing phosphorylation of MEK (65). Although further studies are necessary to elucidate the precise mechanism of ERK inhibition by SU1498, its novel mode of action may provide an impulse toward development of a new class of inhibitors with which to manipulate signaling pathways in human cells.

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