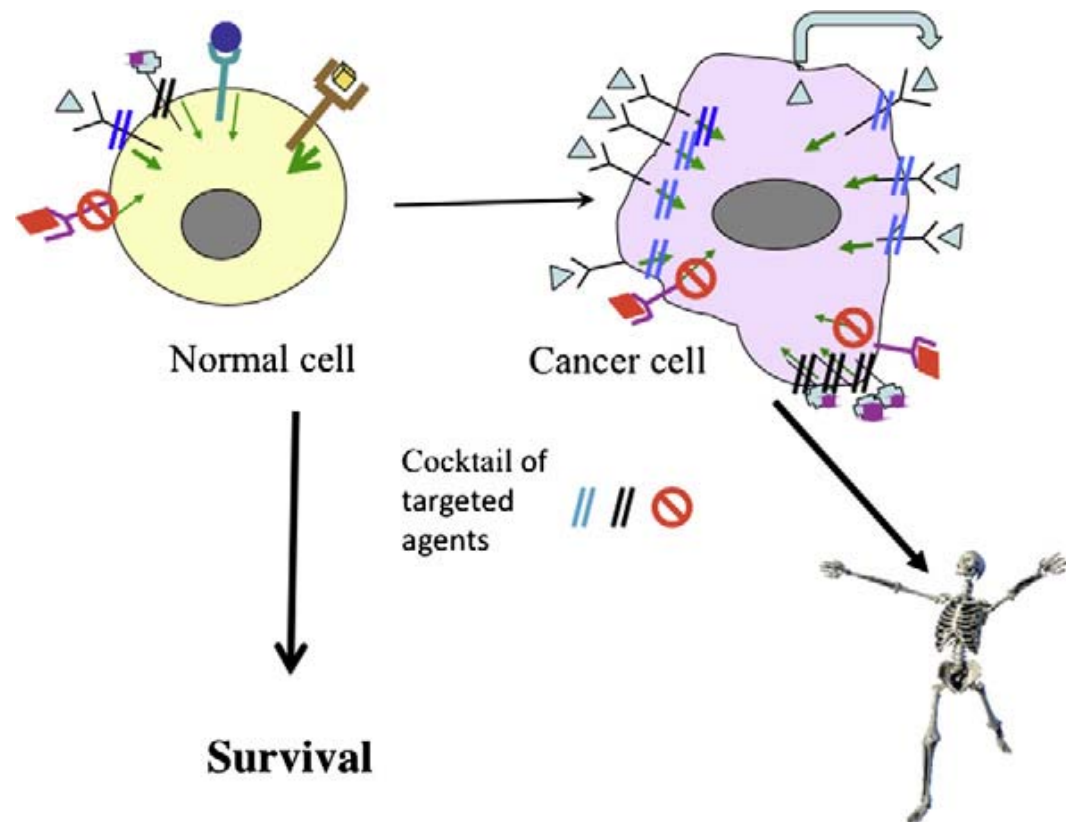


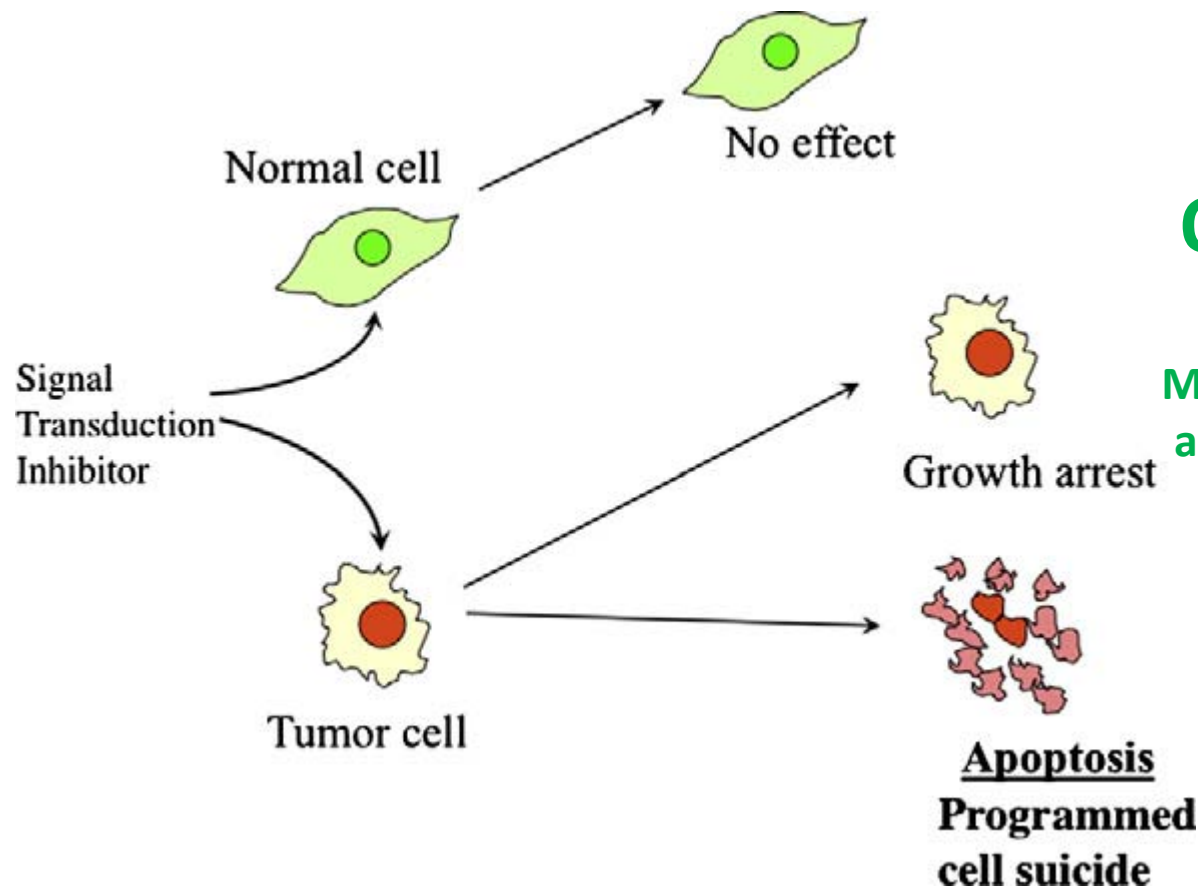
WHY TARGETTING SIGNALLING PATHWAYS ?

- ❑ **Cancer cells are particularly sensitive to stress therefore sensitive to**
 - inhibition of their hyper-activated signaling proteins
 - the re-instatement of lost tumor suppressors.
- ❑ **Complex overlapping signal transduction networks of the healthy cell provide robustness:**
one pathway is inhibited, the effect on the cell is minimized by the presence of alternative, overlapping (“redundant”) pathways, which take over.

- ❑ **Redundancy is reduced in cancer cells, (large number of mutations)**
Cancer cell survival and proliferation depend on a few hyper-active signaling pathways, an Achilles' heel that the therapist can utilize.



THE AIM OF SIGNAL TRANSDUCTION THERAPY.

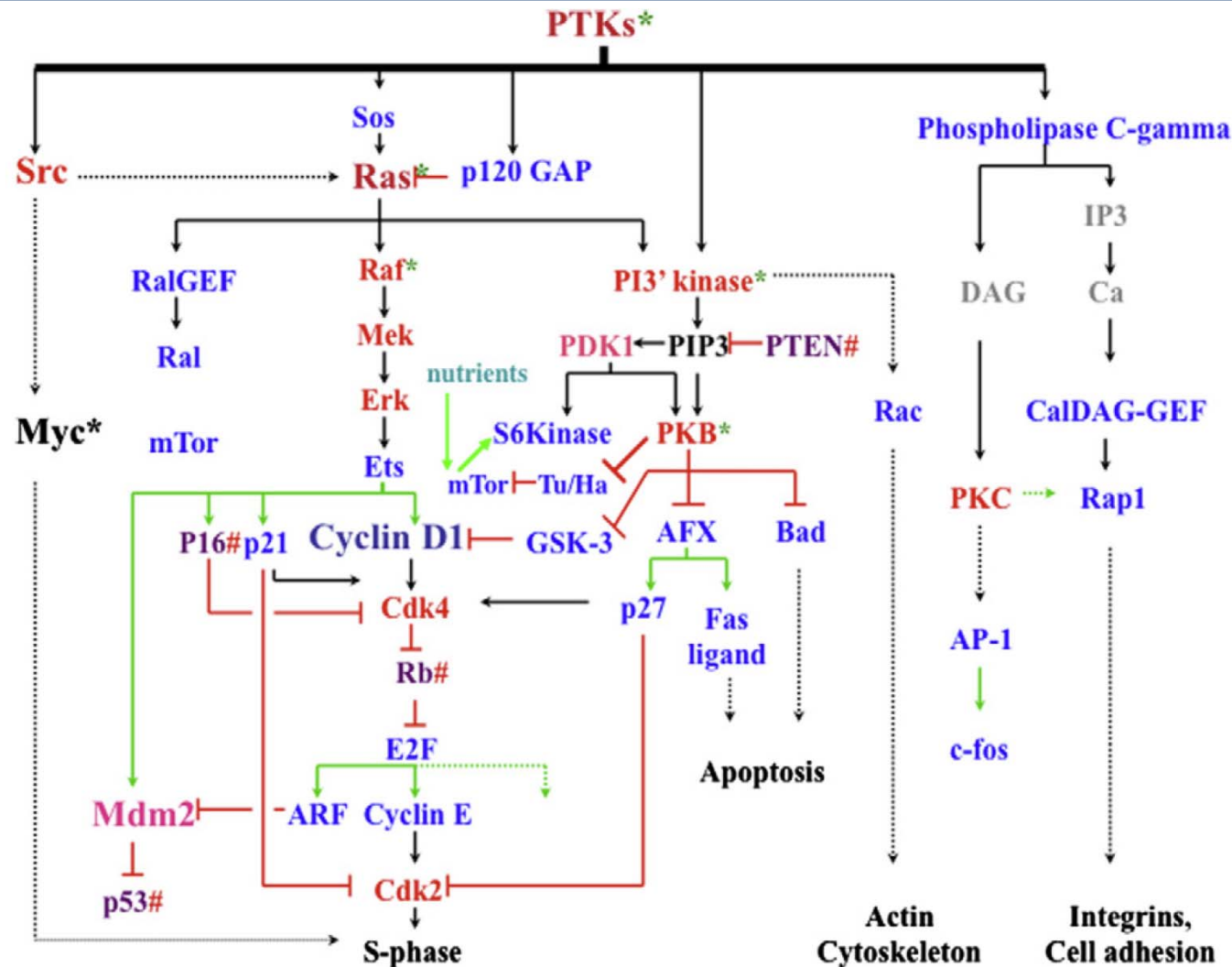


Oncogene addiction

Most cancer cells are not addicted to a single oncogene, nonetheless, the signaling network on which their survival depends is shrunk compared to normal cells.

- ❑ By choosing the right agent or cocktail, the cancer cell will be deprived of signaling elements essential for its survival and undergo irreversible growth arrest or apoptosis.
- ❑ The normal cell will retain its viability, or at most its growth will be arrested and it will be able to resume growth, once the treatment has ended.

PRINCIPLE SIGNAL TRANSDUCTION PATHWAYS.



□ Highly simplified scheme showing the major signaling elements downstream of the receptor protein tyrosine kinases. Possible points of therapeutic intervention are indicated by green asterisks (for oncoproteins) and red hash marks (for tumor suppressors).

PROTEIN KINASE INHIBITORS

- ❑ One third of cellular proteins are phosphorylated
- ❑ Many of the 518 protein kinases are Ser/Thr kinases and only 91 are protein tyrosine kinases (PTKs).
- ❑ The 91 PTKs and a subset of Ser/Thr kinases are involved in cellular signaling. No Ser/Thr kinase inhibitors have entered the clinic, except for the non-selective B-Raf/VEGFR2 kinase inhibitor, BAY 439006/Sorafenib.
- ❑ **A dozen protein tyrosine phosphorylation inhibitors** (tyrphostins/TKI) have already made it to the clinic, where Gleevec is the most successful so far.

The selectivity of protein kinase inhibitors

A number of inhibitors were initially designed to be selective protein kinase inhibitors but turned out to be non-selective !

Ex: Sorafenib was initially identified as a selective Raf inhibitor, but then it was found that its clinical utility was due to its VEGFR2/PDGFR/FGFR inhibitory activity!

The drug was not effective in clinical studies with metastatic melanoma, where 66% of cases express an oncogenic mutation in the Ser/Thr kinase, B-Raf , but is of use against tumors that depend on angiogenesis driven by VEGFR2/PDGFR/FGFR.



❑ Many proponents of “**multi-targeted**” agents surfaced, and **multi-targeting has become a pet**

ideology of targeted therapy drug designers and a popular topic in meetings. Indeed, inhibitors that target two kinases at the same time have been designed and synthesized.

❑ However hitting many targets is most likely the cause of the toxic effects seen for these agents.

Much better to utilize two highly selective inhibitors

ATP competitive inhibitors vs ATP non-competitive inhibitors

Hitting the highly conserved ATP site of a given kinase is likely to hit other protein kinases, as well as some of the 2000 other kinases that phosphorylate substrates other than proteins.

❑ ATP competitive kinase inhibitors are derived from structurally diverse chemical scaffolds, although they share several common characteristics. Most are hydrophobic in nature, which enables them to dock into the non-polar ATP binding site. In addition they have substituents that recognize residues responsible for coordination of the ATP.

❑ **ATP non-competitive inhibitors include substrate competitive inhibitors and allosteric inhibitors**, which bind outside the active site of the enzyme. Tyrphostins that are substrate or bi-substrate competitive are known (Posner et al., 1994).

No substrate competitive inhibitors have yet entered clinical development, so it is not possible to compare them to the ATP mimics that are already in the clinic

❑ No Substrate competitive inhibitors are more selective than ATP competitive inhibitors, since the substrate binding site is much less conserved than the ATP binding site (peptide-based substrate mimics).

SUNITINIB

(SU11248, Sutent TM ; Pfi zer, Inc.)

❑ **advanced renal cell carcinoma (RCC) and for gastrointestinal stromal tumors (GISTs) after disease progression or intolerance to imatinib mesylate (IM) therapy**

❑ **kinase inhibitor of vascular endothelial growth factor receptors :**

VEGFRs 1, 2, and 3

platelet –derived growth factor receptors (PDGFRs α and β)

stem – cell factor receptor (KIT),

FMS - like tyrosine kinase 3 (FLT3),

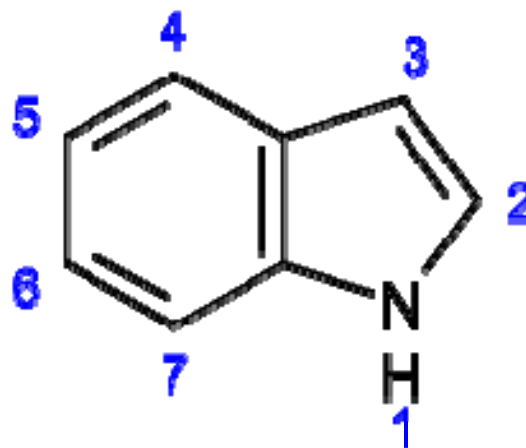
colony – stimulating factor 1 receptor (CSF1R)

glial cell line - derived neurotrophic factor receptor (Rearranged during Transfection; RET)

SUNITINIB DESIGN

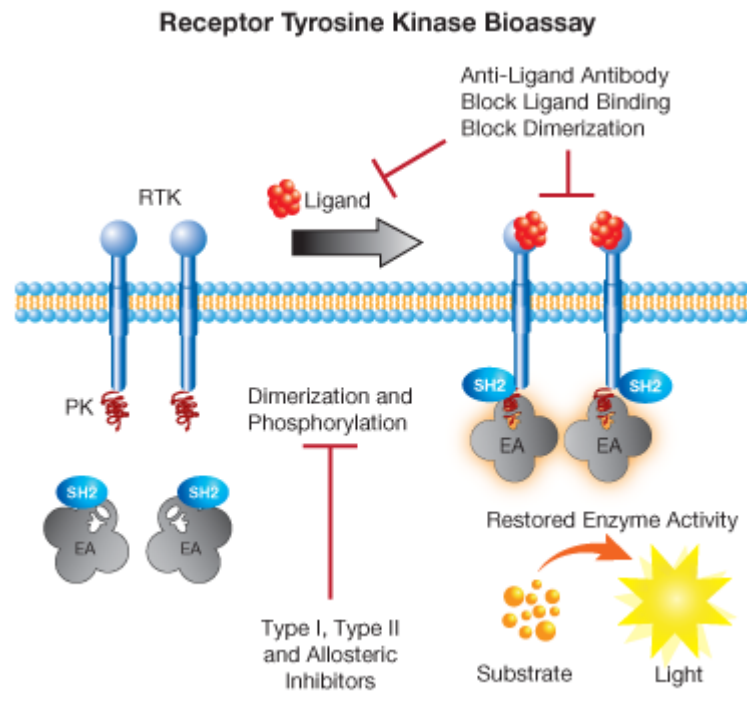
Indoline alkaloids constitute a large class natural product. Their actions have been known for ages

Aztecs used the psilocybin mushrooms which contain alkaloids psilocybin and psilocin.



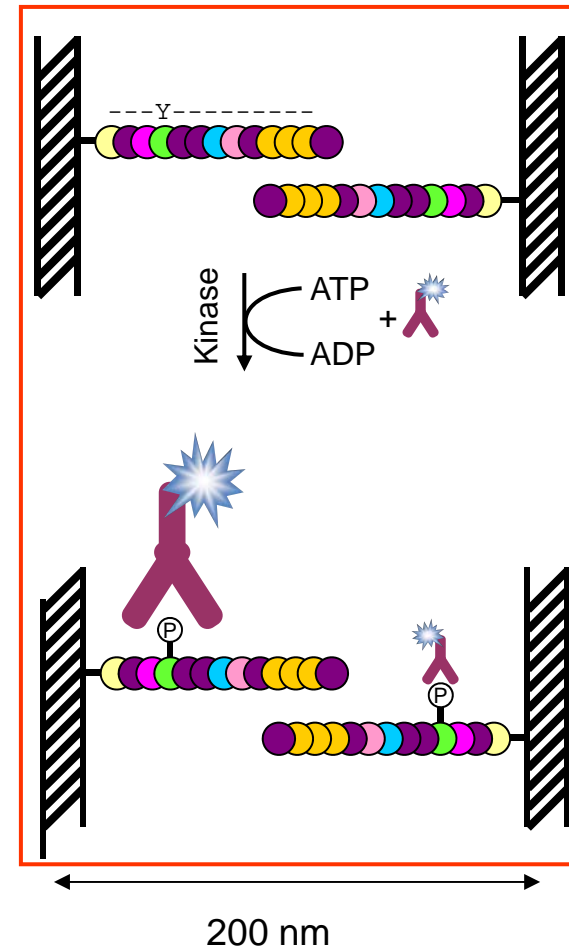
❑ The first chemicals from natural sources were found to be nonselective inhibitors of protein kinases but served as a starting point for novel synthetic chemistry.

RTK INVITRO ASSAY : Biochemical cell based assay



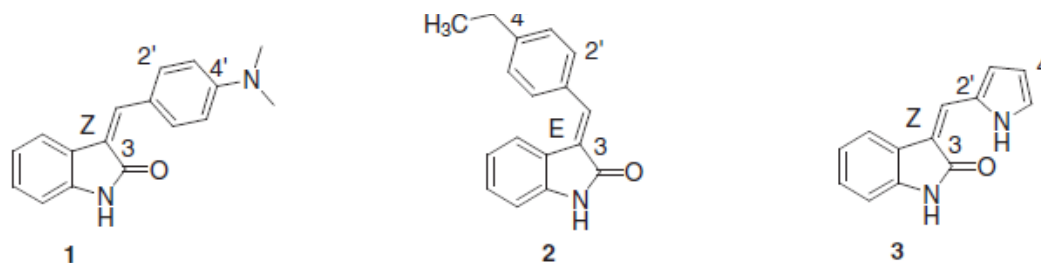
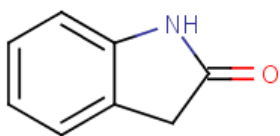
In this PathHunter assay approach, a small peptide epitope (ProLink™) is expressed recombinantly on the intracellular C-terminus of the Receptor Tyrosine Kinase. One of the many different partner proteins containing SH2 domains is co-expressed with a larger sequence, termed enzyme acceptor (EA).

Pam gene technology



In 1994, the drug discovery program company (SUGEN, South San Francisco) targeting tumor angiogenesis by inhibiting VEGFR catalytic activity

A high – throughput screen identified the indolin - 2 - one chemotype with a biochemical PDGFR protein kinase assay.



Compound ID	Inhibition of Cellular Tyrosine Kinase Activity (IC ₅₀ , μM)				
	PDGF	FLK1	EGFR	HER2	IGFR
1	19.4	0.8	>100	>100	>100
2	24.2	5.2	18.5	16.9	10.0
3	12.0	0.39	>100	>100	>100

Figure 1.1. Structures and biological evaluations of the three lead 3-substituted indolin-2-ones (Sun et al., 1998).

- ❑ Both 1 and 3 were found to be potent and selective inhibitors of VEGFR, whereas 2 was found to be nonselective for RTK inhibition.
- ❑ indolin - 2 - ones demonstrated relative specificity in cells comparing VEGF to FGF signaling in cultured human umbilical vein endothelial cells (HUVECs) and comparing PDGF to EGF signaling in cultured fibroblasts.
- ❑ A diverse set of substituted indolin - 2 - ones were designed (Sun et al., 1998, 1999), indolin - 2 - ones with a C - 3 pyrrole substitution demonstrated a better in vitro profile than corresponding compounds with aryl or heteroaryl substitutions

SU5402 as *Z* isomer in NMR solution (Sun et al., 1998).

Relative potency and selectivity for inhibition of VEGFR2 sensitive to the *E/Z* configuration, determined by the nature of substitutions at C - 3 position of the indolin - 2 – ones?

The association of these inhibitory activities with the *Z* isomeric forms was also found to be in agreement with co - crystal studies using 4 and SU5402 bound in the ATP - binding site of the FGFR1 (Mohammadi et al., 1997), which shares high amino acid sequence homology for the ATP - binding pocket with VEGFR2 (see Figure).

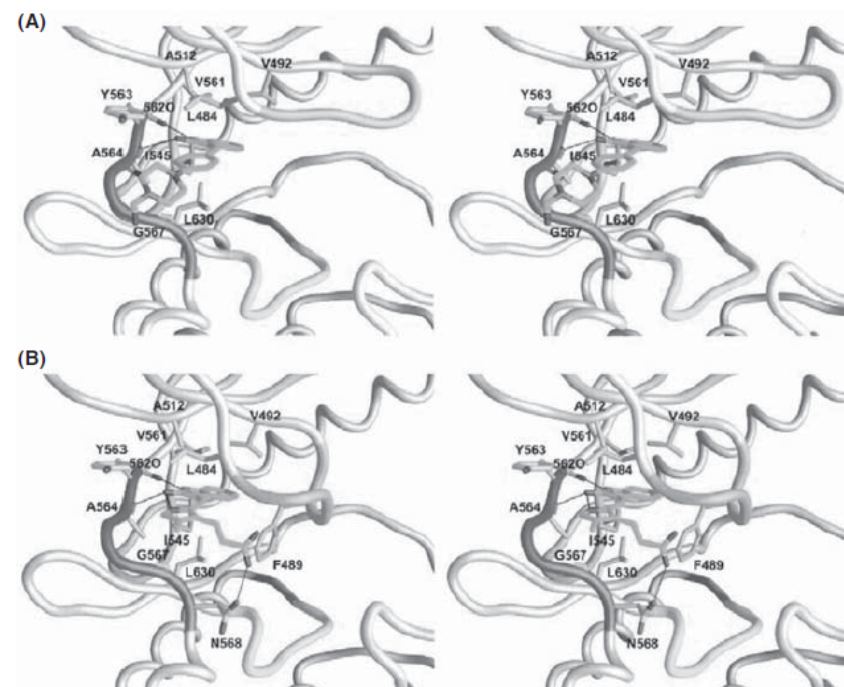
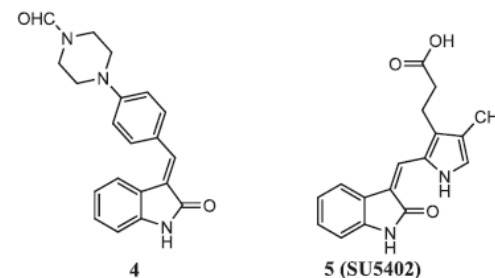


Figure 1.2. Co-crystal structure of SU5402 and compound 4 in FGFR1 kinase domain (Mohammadi et al., 1997). Stereoviews of the inhibitor binding sites. The side chains of residues that interact with the inhibitors are shown. Carbon atoms of the inhibitor and FGFR1K are green and orange, respectively; oxygen atoms are red and nitrogen atoms are blue. Selected hydrogen bonds are shown as black lines. (A) FGFR1K-4. (B) FGFR1K-SU5402. (See color insert.)

□ These structural studies demonstrated the probable molecular mechanism of action for this type of molecule as an ATP - competitive inhibitor and provided additional binding site information for lead optimization.

SU5416: key intermediate

Among the initial set of pyrrol - substituted compounds, SU5416 (Figure 1.3) demonstrated potent and **selective in vitro inhibitory activity toward VEGFR2 and broad spectrum of antitumor activity after daily intraperitoneal administration** (Fong et al., 1999). This was consistent with previous observations with VEGFR2 dominant - negative mutants (Millauer et al., 1996).

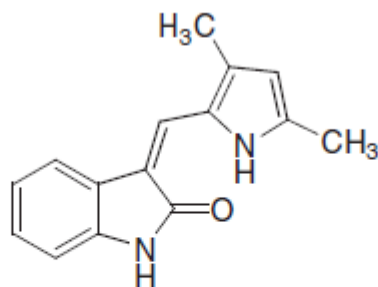


Figure 1.3. SU5416 structure.

TABLE 1.1. Inhibition of Ligand-Dependent Cellular Activities of SU5416

<i>Ligand-Dependent Phosphorylation of RTKs in NIH 3T3 Cells IC₅₀ (μM)</i>			
Flk1	PDGFR	FGFR	EGFR or IGF-R1
1.04 ± 0.53	20.26 ± 5.2	>100	>100
<i>Ligand-Dependent Mitogenesis of HUVEC IC₅₀ (μM)</i>			
VEGFR	FGFR		
0.04 ± 0.02	50		

Source: Fong et al. (1999).

Efficacy of SU5416 in Solid Tumor Xenografts in Mice

Cell Line	Tumor Type	Implant $\times 10^6$ Cells/Animal	Percentage Inhibition @ (days)	<i>P</i>
A375 (human)	Melanoma	3	85 (38)	0.0005
A431 (human)	Epidermoid carcinoma	2.5	62 (20)	0.0006
Calu-6 (human)	Lung carcinoma	7.5	52 (25)	0.031
C6 (rat)	Glioma	0.5	54 (18)	0.001
LNCAP (human)	Prostatic carcinoma	3	62 (43)	0.01
EPH4-VEGF (murine)	Mammary carcinoma	0.5	44 (21)	0.00001
3T3HER2 (murine)	Fibrosarcoma	5	32 (23)	0.046
488G2M2 (murine)	Fibrosarcoma	5	71 (13)	0.0004
SF763T (human)	Glioma	0.5	23 (21)	NS ^b
SF767T (human)	Glioma	0.5	0 (21)	NS

^aVarious tumor cells were implanted subcutaneously in the hind flank region of 8–12 week-old BALB/c *nu/nu* female mice. Animals were treated once daily with a 50 μ L IP bolus injection of SU5416 at 25 mg/kg/day in DMSO or DMSO alone for the indicated number of days (in parentheses) beginning 1 day after implantation. Tumor growth during the treatment period was monitored by measuring the tumor mass on the animals using venier calipers. Tumor volumes were calculated as the product of length \times width \times height. The percentage of inhibition of tumor growth compared with the vehicle-treated control group was calculated on the indicated days after implantation. *P* values were calculated by comparing mean tumor size of the treated group against mean tumor size of the vehicle control group using Student's *t* test.

^bNS, not significant.

Source: Fong et al. (1999).

Broadening the kinase selectivity spectrum of indolin -2 - one to encompass additional class III and V RTKs.

Diversification at the C - 4 ' position on the pyrrol ring of SU5416.

❑ Previous **SAR** analysis showed that modifications at the C - 4 ' position could lead to compounds with different kinase inhibition profiles for the VEGFR2 and PDGFR β RTKs. In this regard, the neutral SU5416 (Fong et al., 1999) was a potent and selective inhibitor for VEGFR2 while acidic SU6668 (Laird et al., 2000) also inhibited PDGFR β .

❑ The **co - crystal structure of SU6668 in the catalytic domain of the FGFR1 kinase** (Laird et al., 2000) revealed that the substitution at the C - 4 ' position on the pyrrole ring was positioned close to the opening of the binding pocket and could be exposed to solvent.

Thus substitution at this position might serve as a handle for improving pharmaceutical properties of the indolin - 2 - ones. Based on this analysis, various basic side chains were introduced at the C - 4 ' position of SU5416.



Among these new analogs, SU11248 (Figure 1.8) (Sun et al., 2003) was identified and exhibited the most optimal overall profile in terms of potency for the intended receptor tyrosine kinase targets, solubility, protein binding, in vivo pharmacokinetic properties, and antitumor efficacy.

In conclusion

❑ Failed after entering clinical trials owing to limited solubility and high toxicity, and SU6668 had inadequate pharmacokinetic properties for clinical development.

❑ Sunitinib resulted from many years of iterative chemistry and pharmacologic testing.

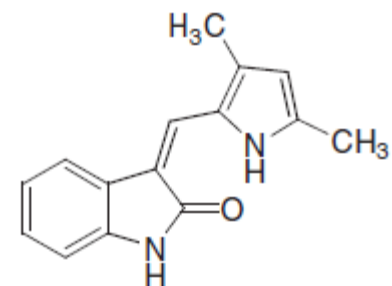
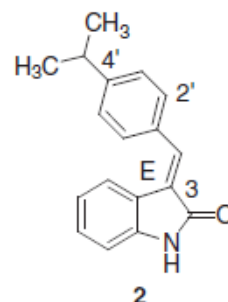
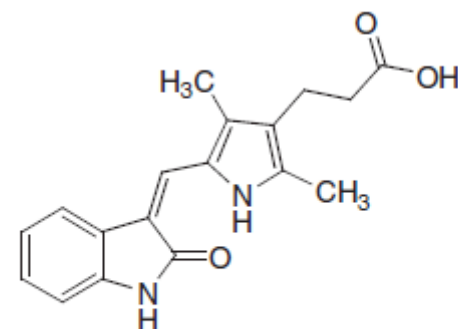
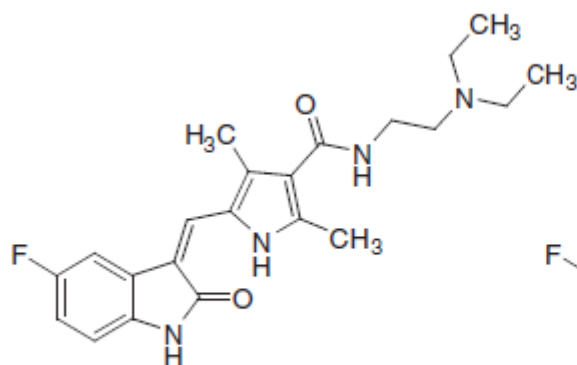


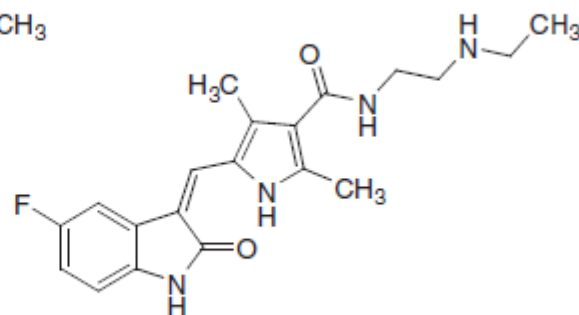
Figure 1.3. SU5416 structure.



SU6668

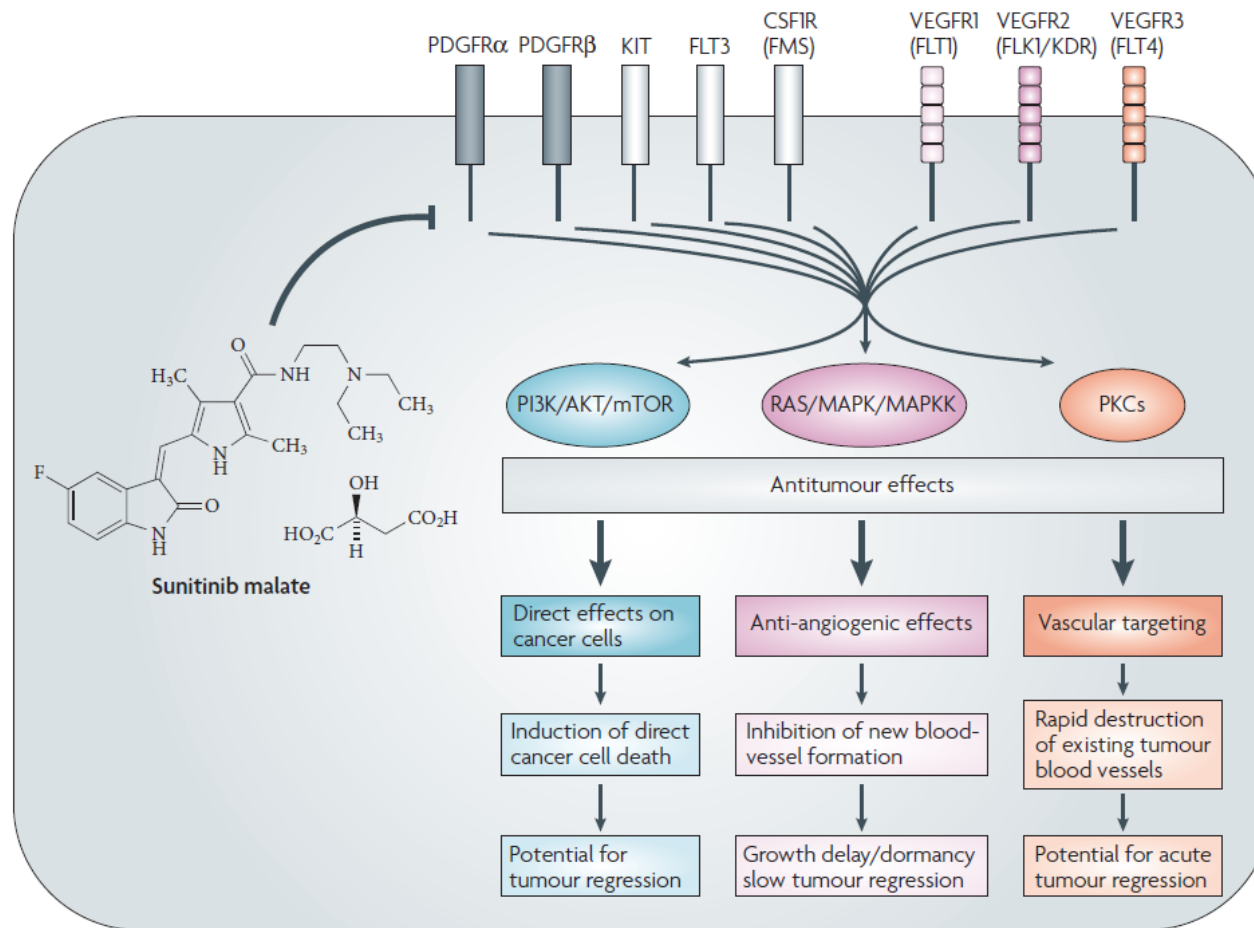


SU11248 (sunitinib)



SU12662 (metabolite of SU11248)

SIGNALLING AND PHYSIOLOGICAL PROPERTIES OF SUNITINIB



The final antitumour effects may be classified as follows:

- ❑ Direct cytotoxic effects on tumour cells by induction of cell death;
- ❑ Anti-angiogenic effects leading to growth delay and/or tumour regression by cytostatic inhibition of new blood-vessel formation;
- ❑ Vascular disruption by inhibition of existing VEGF/VEGFR-dependant tumour blood vessels leading to central tumour cell necrosis, and cavitation that may be associated or not with tumour regression.

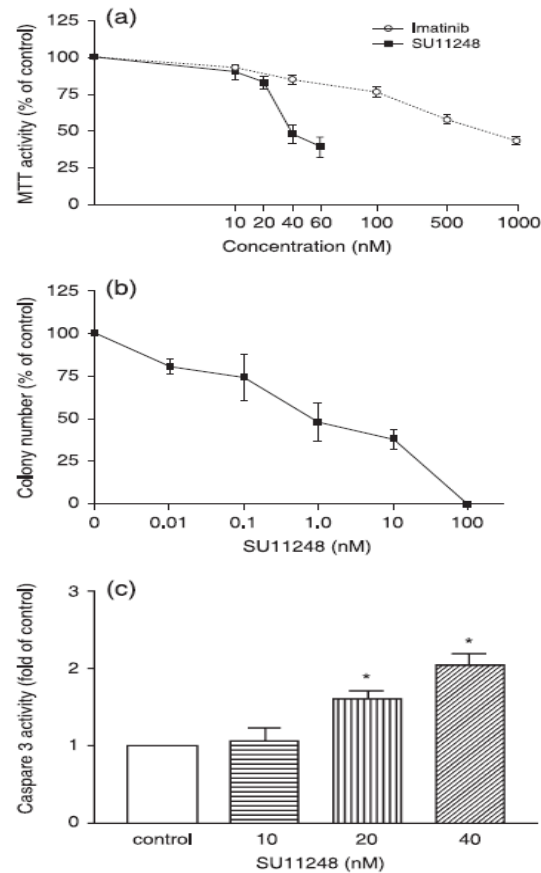
**SIMULTANEOUS INHIBITION OF VEGFR , PDGFR , AND KIT
(SCFR) RTK s ESTABLISHED A UNIQUE MECHANISM OF ACTION
FOR SU 11248**

Table 1 | Summary of the biological effects of sunitinib against target receptors

Receptor	Biochemical K_i (μ M)	Cellular IC_{50} (μ M)		References
		Receptor phosphorylation	Proliferation	
VEGFR1*	0.002	Not determined	Not determined	Pfizer, unpublished observations
VEGFR2 [†]	0.009	0.01	0.004	20
VEGFR3 [§]	0.017	Not determined	Not determined	Pfizer, unpublished observations
PDGFR β	0.008	0.01	0.039	20
PDGFR α	Not determined	Not determined	0.069	20
KIT	0.004	0.001–0.01	0.002	28
FLT3 (wild-type)	Not determined	0.25	0.01–0.05	30
RET	Not determined	0.05	0.05	Pfizer, unpublished observations
CSF1R	Not determined	0.05–0.1	Not determined	29

*Also known as FLT1. [†]Also known as FLK1 or KDR. [§]Also known as FLT4. CSF1R, colony stimulating factor 1 receptor; FLT3, fms-related tyrosine kinase 3; KIT, stem-cell growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

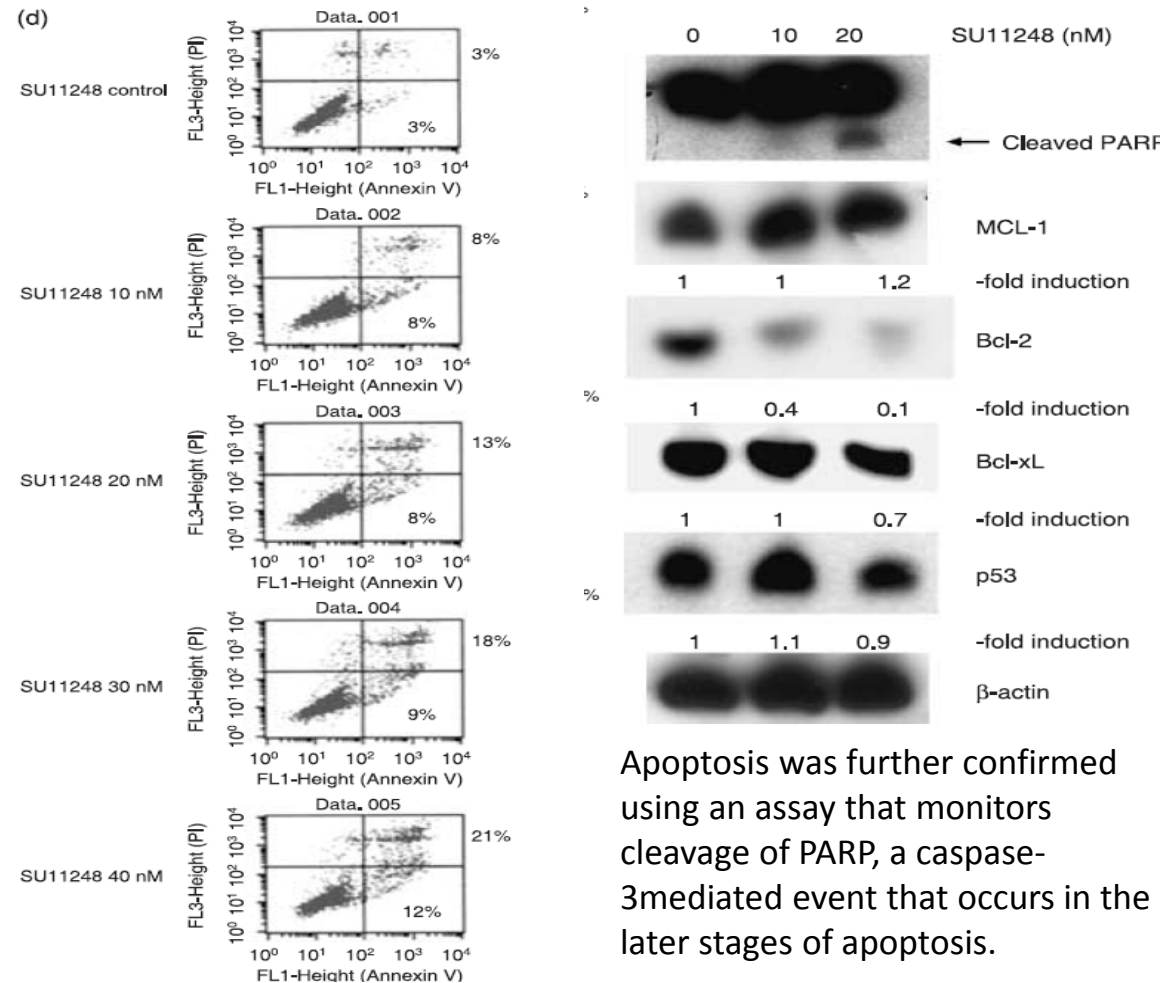
SU11248 induces growth arrest and apoptosis of GIST-T1 cells



a- Viability : MTT test

b- Clonogenicity Colony formation

c- SU11248 at either 20 or 40 nM increased caspase-3 activity approximately 1.6-fold and 2.1-fold, respectively, compared to control cells



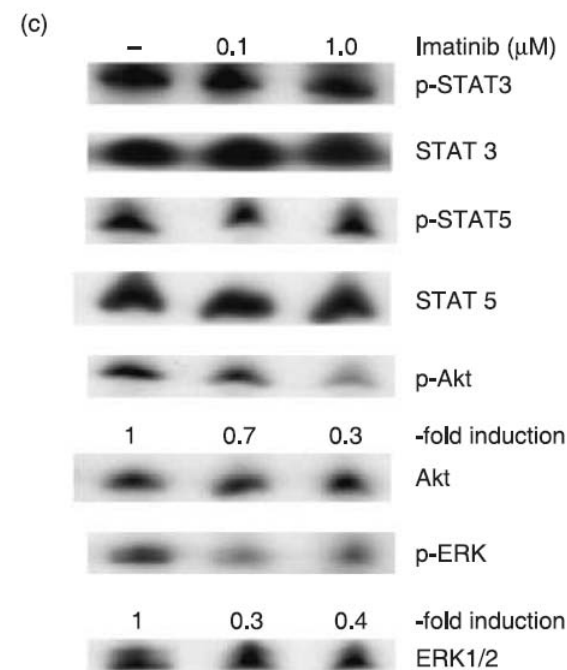
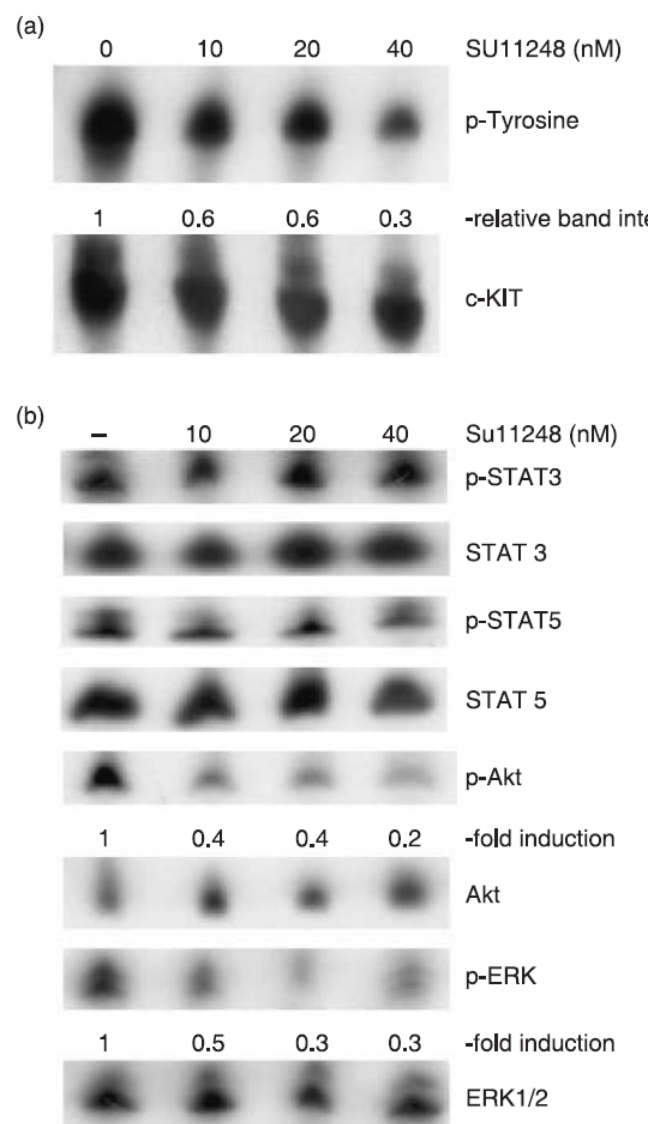
Apoptosis was further confirmed using an assay that monitors cleavage of PARP, a caspase-3 mediated event that occurs in the later stages of apoptosis.

SU11248 dosing at 10 or 20 nM for 48 h significantly downregulated the levels of Bcl-2, although other Bcl-2 family members, including Mcl-1, Bcl-xL and p53, were not affected.

SU11248 inhibits tyrosine phosphorylation of c-KIT in GIST-T1 cells

Effects of SU11248 on downstream effectors of c-KIT in GIST-T1 cells. GIST-T1 cells aberrantly expressed the phosphorylated forms of STAT3, STAT5, Akt and ERK (Fig. 2a-2b).

Blockade of PI3K/Akt/mTOR signaling potentiates the effect of SU11248 in GIST-T1 cells



However, SU11248 did not affect phosphorylated forms of STAT3 and STAT5 (Fig. 2B). Similarly, imatinib (0.1 or 1 μM, 48 h) blocked p-Akt and p-ERK, but not p-STAT (Fig. 2c).