



Trip Report for

**“Chemistry in Cancer Research: A Vital Partnership
in Cancer Drug Discovery and Development”**

New Orleans, LA

February 8-11, 2009

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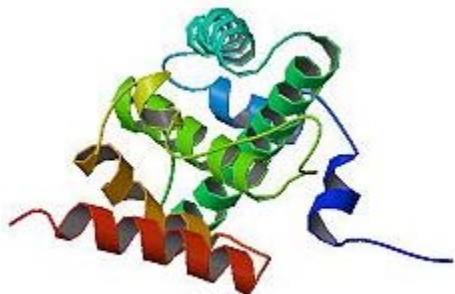
Abstract: *The joint conference presented by the American Association for Cancer Research (AACR) and the American Chemical Society (ACS) was held at the Westin New Orleans Hotel in New Orleans, LA. Forty-four seminars were presented covering several topics: cancer target ID and validation, lead discovery (chemical libraries, screening, hit validation, natural products, chemoinformatics), lead optimization (case histories), cancer drug targeting, drug delivery and drug disposition, drug discovery and development. This report summarizes some of the recent research in medicinal and biochemistry by major pharmaceutical companies and academia.*

“Disrupting the Rb-Raf-1 Protein-protein Interaction: A New Strategy for Anticancer Drug Design”

Nicholas J. Lawrence, Moffitt Cancer Center and Research Institute, Tampa, FL

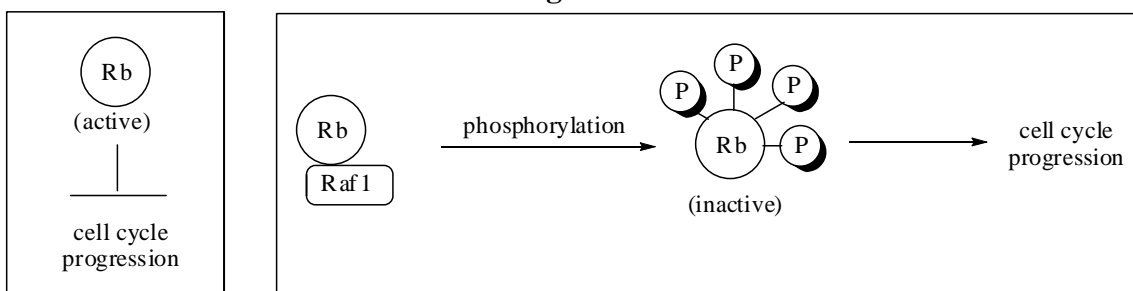
It is well known that the retinoblastoma tumor suppressor protein (Rb, Figure 1) plays a key role in regulating cell cycle progression and its inactivation is necessary for entry into S phase.

Figure 1

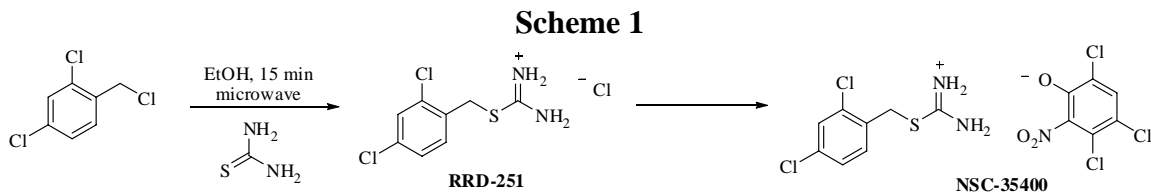


Active Rb inhibits cell cycle progression (Figure 2). The inactivation of Rb is achieved when growth factor stimulation of cells leads to the binding of Raf-1 kinase to Rb, which then undergoes phosphorylation to an inactive form. Hence, the transition from G₁ phase to S phase is enabled, allowing for normal cell cycle progression. It has been shown that the Rb regulatory pathway is compromised in most human cancer cells. Disruption of the Rb-Raf-1 binding may assist in controlling cancerous cells.

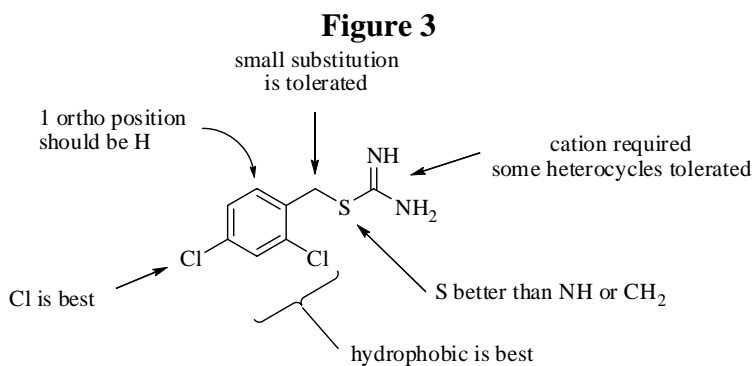
Figure 2



A series of 2-(2,4-dichlorobenzyl)isothiuronium analogues have been developed as cancer therapeutics (Scheme 1). It has been suggested that activity of these drugs is related to the cation. ELISA analysis showed that the Rb/Raf-1 interaction was disrupted by NSC-35400 with an IC₅₀ of 81 nmol/L and RRD-251 with an IC₅₀ of 77 nmol/L. At concentrations of 20 μmol/L, ELISA analyses showed that these inhibitors were also selective for Rb/Raf-1 interaction over Rb/E2F1, Rb/HDAC1, Rb/prohibitin, and Raf-1/Mek interactions.



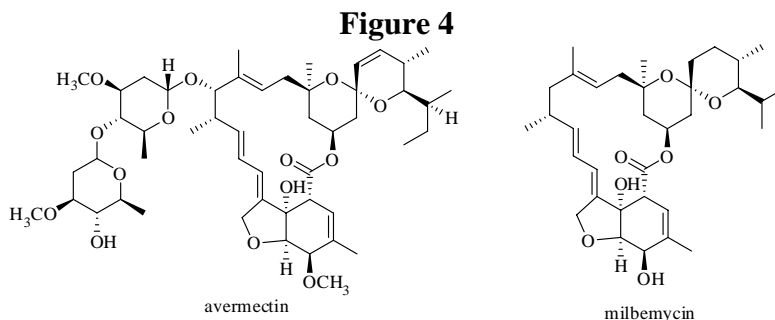
Extensive SAR was developed (Figure 3) and the orally bioavailable analogue RRD-251 was shown to inhibit (150 MPK in A549 mouse xenograph PO at $237 \pm 67 \text{ mm}^3$) the proliferation of many tumor cell lines in an Rb-dependant manner.



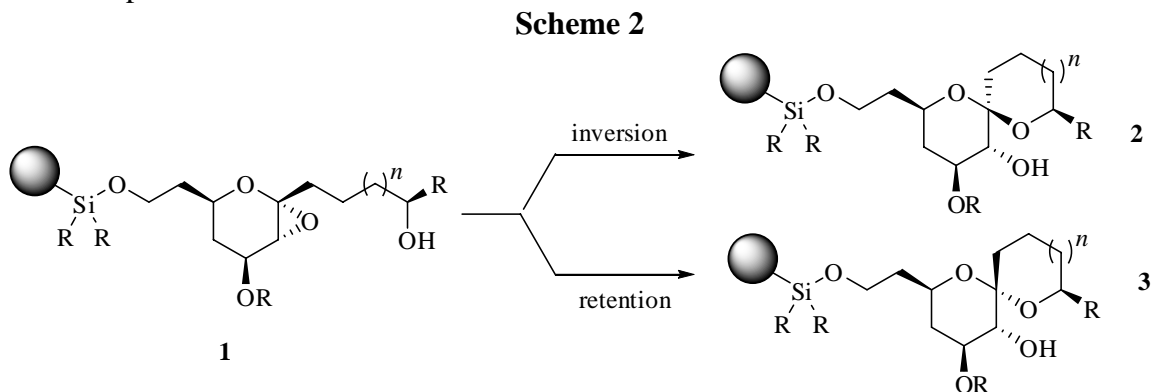
“Natural Product-based Libraries for Chemical Biology and Drug Discovery”

Derek S. Tan, Memorial Sloan-Kettering Cancer Center, New York, NY

A recent study showed that available drugs address only 207 current drug targets or about 1% of targets within the human genome. Nearly 50% of current drugs target GPCRs, nuclear receptors, and ion channels. It is estimated that fifteen times more targets may be therapeutically relevant. Natural products are known to address a wider range of targets than traditional synthetic drugs. Using structural motifs found in biologically active natural products, researchers at Memorial Sloan-Kettering Cancer Center hope to develop libraries which may address a broader scope of therapeutic targets. The spiroketal motif is a privileged structure which induces structural rigidity and provides stereochemical diversity. Natural product examples (Figure 4) include avermectins, a group of broad-spectrum antiparasitic antibiotics, and milbemycins, a family of antibiotics with insecticidal and acaricidal activity.



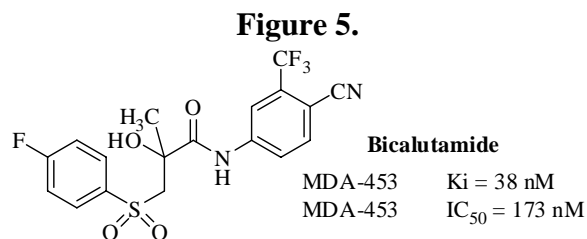
As shown in Scheme 2, using a resin-bound glycol epoxide linked via silylether **1**, careful selection of cyclization conditions could provide either the inversion product spiroketal **2** or the retention product **3** spiroketal. By employing methanol to induce cyclization at $-63\text{ }^{\circ}\text{C}$, the inversion (kinetic) product **2** was obtained in 92% yield. After significant experimentation, $\text{Ti}(i\text{-PrO})_4$ as an additive enabled nearly exclusive access to the retention product **3**.



“The Discovery of BMS-641988: A Novel Androgen Receptor Antagonist for the Treatment of Prostate Cancer”

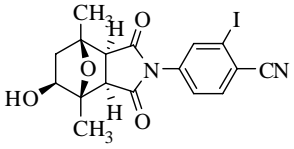
Aaron Balog, *Bristol-Myers Squibb Pharmaceutical Research and Development, Princeton, NJ.*

Carcinoma of the prostate (CaP) is the most frequently diagnosed malignancy in men in the U.S. and the second leading cause of cancer-related death among U.S. males. Over 218,890 new cases and 27,050 deaths due to CaP were predicted for 2007. Androgen ablation by chemical or surgical castration or use of steroid synthesis inhibitors, LHRH analogues, or antiandrogens is typically the treatment of choice for advanced cases of CaP. Despite treatment, nearly half of the patients eventually progress to fatal androgen independent CaP (AI-CaP). It has been shown that reactivation of the androgen receptor (AR) signaling pathway is the leading cause for AI-CaP, hence drugs that act as antagonists of the AR may be useful in the treatment of this disease. Researchers at BMS developed novel antagonists of AR, using MB-MDA-453 for a whole cell competitive binding and transactivation assay (*in vitro*) screen. Good leads were then subjected to an immature rat prostate weight PK/PD model (IRPW, PO, QD \times 4 days), from which the most promising compounds were then screened in the human cancer (CaP) xenograft model CWR-22LD1, implanted in nude mice. The “gold standard” used for evaluation was a clinical antiandrogen Bicalutamide (Figure 5) with potent activity.

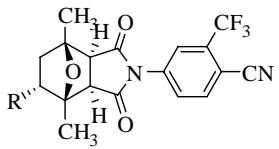


SAR of a [2.2.1]-oxobicyclic imide core is depicted in Table 1. An early lead, **BMS-591305**, was found to have good activity but contained a labile iodobenzene moiety. Further development provided analogue **BMS-641988**, which was synthesized via selective Diels-Alder reaction and has been found to possess improved efficacy in CWR-22LD1 over bicalutamide. This lead compound displayed an AR binding (K_i) of 1.7 nM and an MDA453 IC_{50} of 16 nM, and is currently in Phase 1 studies.

Table 1



BMS-591305
MDA453 K_i = 1.5 nM
MDA453 IC_{50} = 20 nM



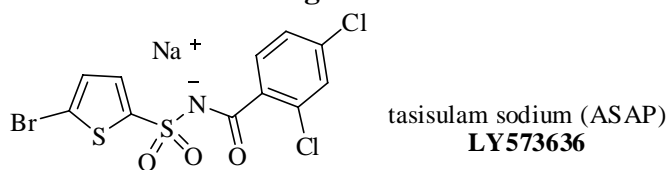
R	Ki (nM)	IC ₅₀	Plasma Exposure (mM)
CH ₂ OH	81	150	
CO ₂ H	1600	>5000	
NH ₂	31	60	0.5
CON(CH ₃)Ph	50	34	12
NHCO(4-F-Ph)	10	19	310
NHCO ₂ iPr	3	9.9	165
NHCO ₂ (CH ₂) ₂ (morpholine)	12	12	19
NHCONHCH ₂ (cyclopropyl)	16	40	251
NHSO ₂ NHCH ₃	50	34	2
NHSO ₂ (morpholine)	7	10	1
NHSO ₂ CH ₃	3	13	4
NHSO ₂ Et (BMS-641988)	1.7	16	4

“The Discovery and Development of Diaryl-acylsulfonamide (tasisulam sodium, ASAP): A Novel Class of Antitumor Agents for Solid Tumors”

Chuan Joe Shih, Eli Lilly Research Laboratories, Indianapolis, IN

Researchers at Eli Lilly in conjunction with Wayne State University discovered a novel diarylacylsulfonamide, tasisulam sodium (Figure 6), which displays antitumor activity. A simple soft-agar disk diffusion (SADD) colony formation assay was used as a primary screen to identify compounds that can preferentially kill solid tumor cells (colon HCT116/HCT115, lung, breast, etc.) over both leukemia and normal fibroblast cells *in vitro*. Promising leads were then tested against a broad panel of murine (colon 38, mam16C, mam17/0, colon 26, panc 03, squam cell lung LC-12) and human solid tumor lines (prostate LNCap, prostate PC-3, lung H125, squam lung H165, and colon H116) *in vivo*. With unknown molecular targets, the xenograft efficacy results were used as the key driver for SAR and compound selection.

Figure 6.



It was discovered that Tasisulam exhibited no kinase activity and was shown to induce apoptosis via activation of the mitochondrial cell death pathway. Tasisulam inhibited cellular ATP production and displayed a dose-dependent increase of reactive oxygenated species (ROS). The molecular target is still not yet known, but tasisulam is currently in three different Phase II studies. One study has determined the drug to be 99% albumin bound. To resolve this issue, researchers are testing a tailored dosing schedule.

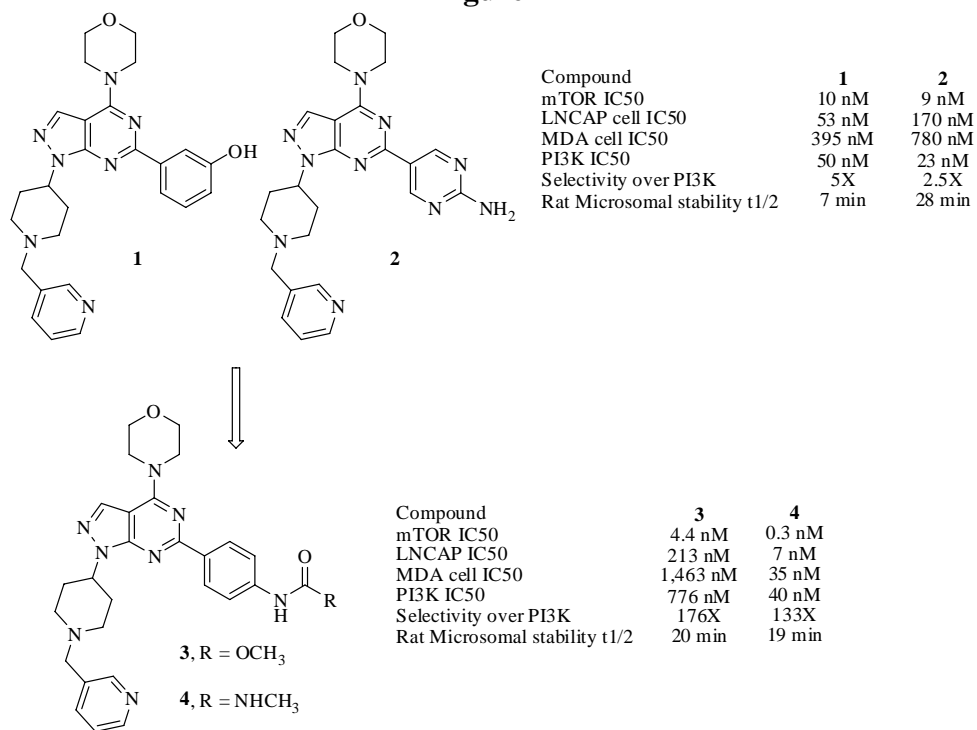
“Synthesis and Anti-Tumor Properties of Pyrazolopyrimidines: Potent, ATP-Competitive, and Selective Inhibitors of the Mammalian Target of Rapamycin (mTOR)”

David J. Richard, Wyeth Research, Pearl River, NY

The serine-threonine kinase mammalian target of rapamycin (mTOR) is a member of the phosphoinositide-3-kinase (PI3K) related PIKK kinase family. This family of kinases play a crucial role in the regulation of cellular growth. In particular, mTOR responds to increased levels of insulin, nutrients, or energy supply by activating downstream proteins leading to proliferation. Inhibition of mTOR with derivatives of the natural product rapamycin have demonstrated efficacy as a clinical anti-cancer treatment. The mTOR signaling pathway involves two distinct complexes, mTOR/raptor (TORC1) and mTOR/riCTOR (TORC2). Rapamycin analogues have been shown to inhibit the TORC1 complex, but have no effect on the TORC2 complex. This is important because the TORC2 complex activates AKT which has been demonstrated to have anti-apoptotic effects. Therefore, the team at Wyeth sought to develop small molecule inhibitors of both TORC1 and TORC2, and that were selective against the membrane-bound lipid kinase PI3K that is upstream of mTOR and shares significant homology.

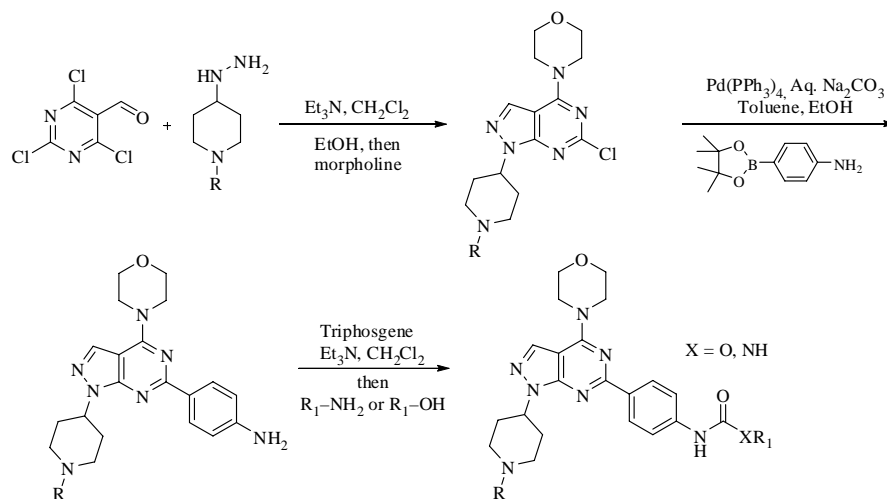
After some initial modification to a screening hit, the Wyeth team identified several promising early lead compounds as shown in Figure 7. Initial compounds such as **1** or **2** displayed good enzyme and cell based potency. However, these compounds suffered from rather poor selectivity against PI3K and in the case of the phenol compound **1**, modest microsomal stability. Incorporation of aryl carbamates such as **3** or aryl ureas like compound **4** gave rise to improved enzyme and cell based potency. Additionally, these compounds had significantly improved selectivity against PI3K.

Figure 7



The synthetic sequences employed to prepare these compounds is depicted in Scheme 3. The synthesis began by condensation of the appropriately substituted piperidine hydrazine with the trichloropyrimidine aldehyde. Subsequent Suzuki-Miyaura cross coupling gave rise to the key aniline intermediate. The aniline could then be easily converted to the desired carbamates or ureas by treatment with triphosgene, followed by the appropriate alcohol or amine.

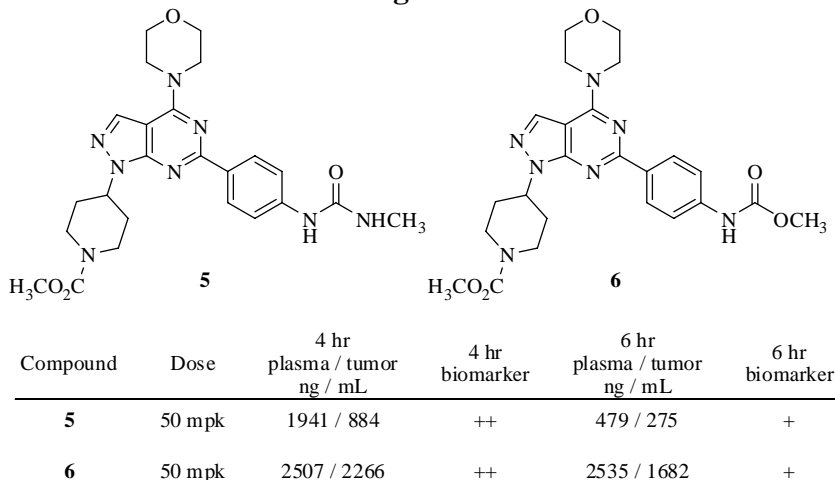
Scheme 3



After developing some early SAR, the team chose to examine both a urea and a carbamate in a nude mouse xenograft study. Compounds **5** and **6** had reasonable cell

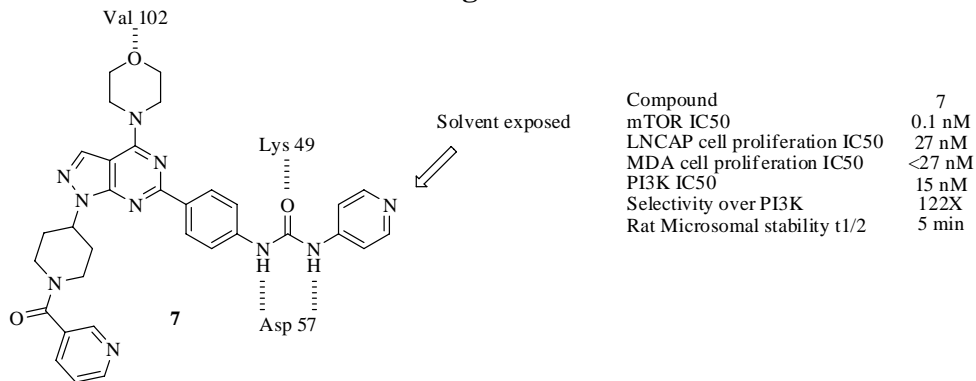
based potency (27-400 nM) and good selectivity against PI3K (278-495X). Shown in Figure 8 are some pharmacokinetic and biomarker results from this early study. The team was pleased to find that both compounds displayed the desired biomarker activity to at least the 6 hour time point. However, a surprising result was found in the efficacy study. It was found that the carbamate **6** was equally as efficacious as the urea **5**, despite there being a ~20 fold difference in *in vitro* cell based potency. This was attributed to the superior PK properties of the carbamate relative to the urea. The team then focused on how to improve the pharmacokinetic properties of the more potent urea derivatives.

Figure 8



A key breakthrough was made when the team was able to obtain a crystal structure of inhibitor **7** bound in the active sight of the enzyme. The team found that there were three key hydrogen bonding interactions (shown in Figure 9): one with the morpholine oxygen atom, and two with the urea. Importantly, the terminal end of the pyridine group was protruding out of the active sight and was somewhat solvent exposed. The chemists felt that this position would provide a handle for incorporation of water solubilizing groups into the compound to improve the PK profile.

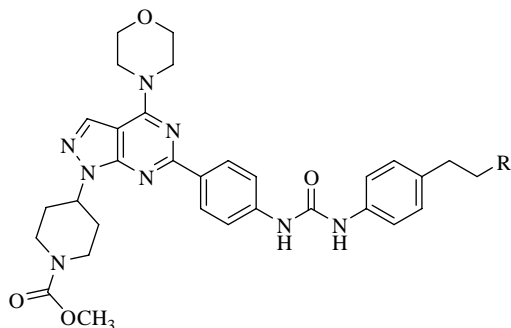
Figure 9



Shown in Table 2 are their SAR results with a series of substituted phenethyl analogues. The team incorporated a number of cyclic and acyclic secondary and tertiary amines in

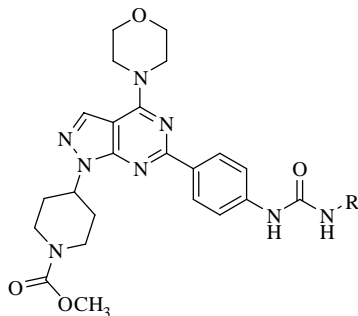
hopes of improving solubility. This SAR lead to several compounds with reasonable aqueous solubilities, however the cell based potencies were typically modest. The researchers then examined incorporation of alcohols or aryl amines as shown in Table 3.

Table 2



Compound	R	mTOR (nM)	PI3K (nM)	Selectivity	LNCAP (nM)	MDA (nM)	Solubility (pH 7, $\mu\text{g/mL}$)	Microsomal stability ($t_{1/2}$, min)
8		0.7	34	49	<0.8	20	53	>30
9		0.6	25	42	0.8	25	33	>30
10		0.7	20	28	1.3	110	61	-
11		0.8	20	27	1.4	60	0	17
12		0.6	40	68	5	180	0	20
13		0.5	7	16	31	420	>100	14
14		0.5	5	9	0.8	300	60	>30
15		0.4	12	31	1	120	22	-

As depicted in Table 3, there was significant improvement in cell based potency by addition of alcohols or aryl amines. The most promising compound was **19**, which possessed excellent cell based potency, good selectivity over PI3K, good microsomal stability, and good aqueous solubility (at pH = 3).

Table 3

Compound	R	mTOR (nM)	PI3K (nM)	Selectivity	LNCAP (nM)	MDA (nM)	Solubility (pH 7, $\mu\text{g/mL}$)	Microsomal stability (t 1/2, min)
16		0.08	6	75	<0.8	<0.8	0	8
17		0.12	10	83	<0.8	1.3	1	8
18		0.3	118	393	0.8	10	0	16
19		0.34	14	42	<0.8	0.8	>100 (pH 3)	>30

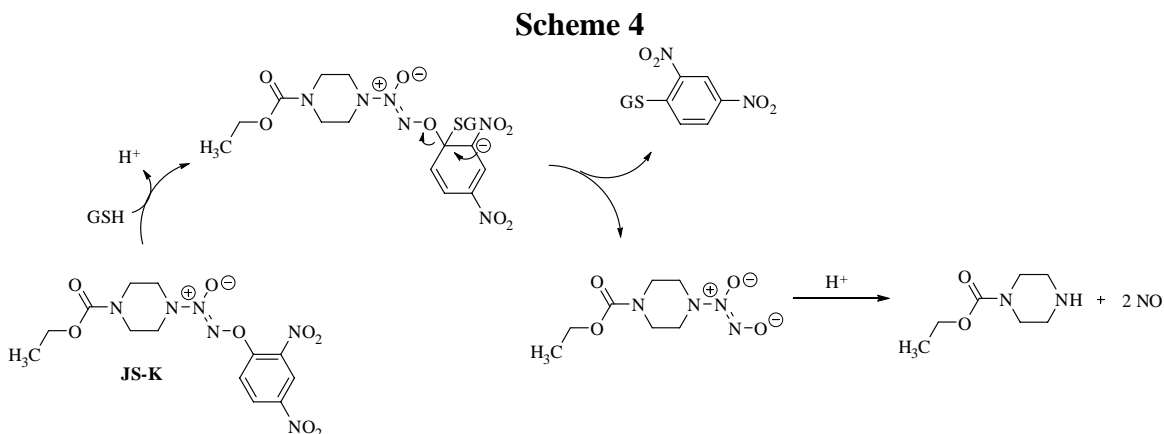
To summarize, the researchers at Wyeth identified a series of ureidophenyl substituted pyrazolopyrimidines as highly potent inhibitors of the mTOR complexes with both TORC1 and TORC2. These compounds also possessed selectivity against the highly homologous PI3K. Early compounds demonstrated efficacy in nude mouse xenograft studies. After examination of the binding mode of their inhibitors, further modification lead to highly cell potent compounds with good aqueous solubility and microsomal stability.

“Structural Analogues of JS-K, an Anti-Cancer Lead Compound”

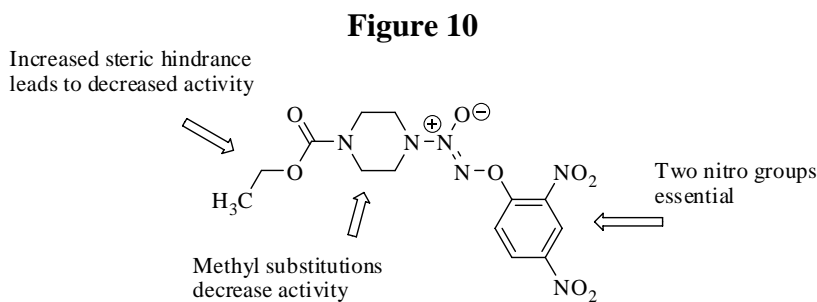
Larry K. Keefer, National Cancer Institute at Frederick, Frederick, MD

Doctor Keefer presented research related to JS-K and structurally related analogues. JS-K is a member of the diazeniumdiolate class of glutathione/glutathione S-transferase-activated nitric acid prodrugs. This class of compounds has shown promise as an anti-cancer treatment through the release of metabolic NO that can mediate cytotoxic activities. The researchers sought to prepare several structural analogues of JS-K and examine their anti-proliferative activity.

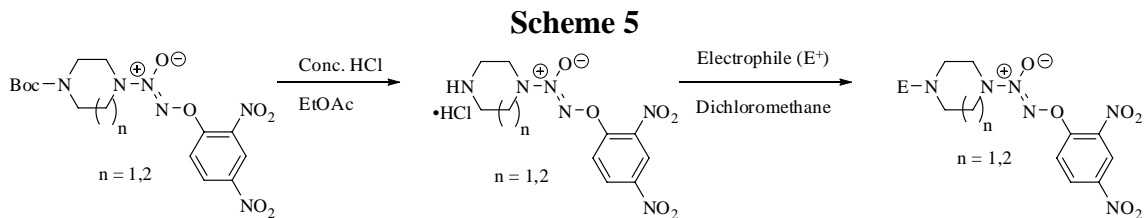
Shown in Scheme 4 is the proposed mechanism for the release of NO from JS-K (or related NO prodrugs). The compound undergoes an addition / elimination reaction mediated by glutathione, ideally in the presence of glutathione S-transferase (which is upregulated in many human tumors). This gives rise to a charged intermediate that in the presence of a proton source should further decompose to yield the piperazine moiety, along with 2 equivalents of nitric oxide.



Some of the early SAR trends are summarized in Figure 10. The team found that the presence of the two nitro groups on the aromatic ring was critical to maintain good levels of NO release. The team also found that large, sterically hindered carbamates lead to decreased levels of nitric oxide release (and corresponding decreases in anti-proliferative activity). Additionally, alkyl substitutions on the piperazine ring were also not tolerated. The researchers then focused the SAR around small carbamates in both the 6-, and 7-membered piperazine compounds.



Shown in Scheme 5 is the synthetic scheme the team used to develop their SAR. The team was able to utilize the known Boc-protected intermediate as the key starting material. This compound was de-protected with acid to give the HCl salt of the piperazine. This salt could then be functionalized with a variety of electrophiles to provide the desired substituted compounds.



A summary of the SAR the NCI team developed is shown in Tables 4 and 5. The team explored a number of small carbamates, ureas, thiocarbamates, and phosphonamides. The chemists found that simple changes such as fluoroethyl or vinyl groups were well tolerated, yielding compounds that provided similar NO release as JS-K, as well as similar cell IC₅₀ values. Chain homologation or changing from a carbamate to other heteroatoms provided generally poorer compounds.

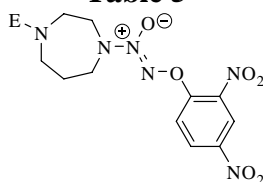
Table 4

Compound	E	% NO release	HL-60 IC ₅₀ (μM)	U937 IC ₅₀ (μM)
1		85	0.5	0.3
2		88	0.4	0.7
3		90	0.3	0.4
4		83	1.1	1.0
5		86	1.2	1.1
6		84	1.6	1.4
7		98	1.4	1.1
8		74	1.9	1.9
9		85	5.7	5.1

The team was able to obtain more promising results when they moved to examine several homopiperazine analogues. The researchers identified several carbamates that provided

similar or superior nitric oxide release compared to JS-K. Additionally, these compounds also had very good cell IC₅₀ values for their anti-proliferative activity (200 – 600 nM). Additionally, the chemists found that there was good correlation between the anti-proliferative activity and the amount of intracellular NO release.

Table 5



Compound	E	% NO release	HL-60 IC ₅₀ (μ M)	U937 IC ₅₀ (μ M)
10		100	0.2	0.3
11		87	0.4	0.5
12		93	0.2	0.3
13		98	0.4	0.6
14		99	0.6	0.6
15		90	0.5	0.5

To summarize, the researchers at NCI explored several new analogues of JS-K as possible new anti-cancer compounds. They found that several homopiperazine carbamates provided similar to superior results in terms of both nitric oxide release, as well as anti-proliferative activity. In addition, the team found good correlation between the amount of intracellular NO delivered and cellular growth inhibition. For further information on this topic, see: (1) Shami *et. al. Mol. Cancer Ther.* **2003**, 2, 409-417; (2) Chakrapani *et. al. Bioorg. Med. Chem.* **2008**, 16, 9764-9771; (3) Shami, *et. al. J. Med. Chem.* **2006**, 49, 4356-4366.