Development of Sphingosine Kinase Selective Inhibitors for Cancer Therapy

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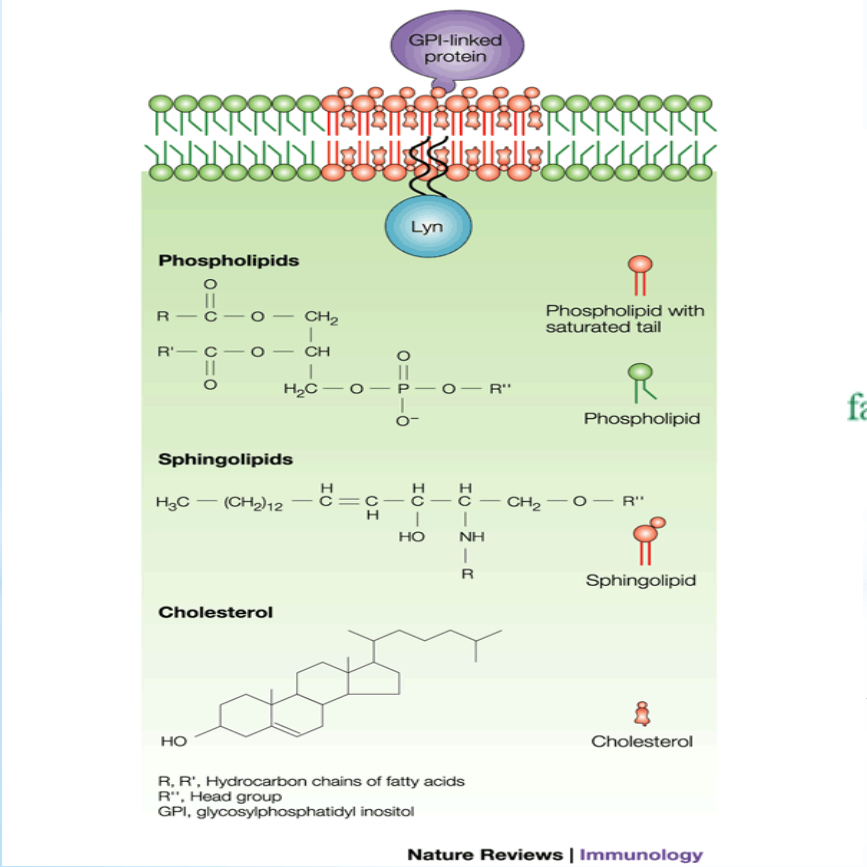
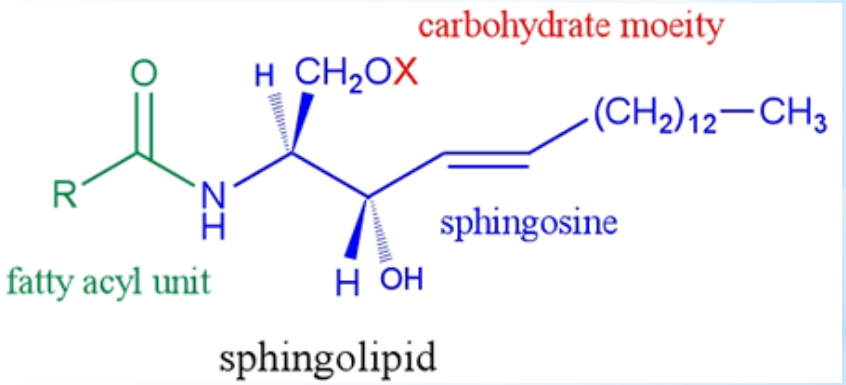
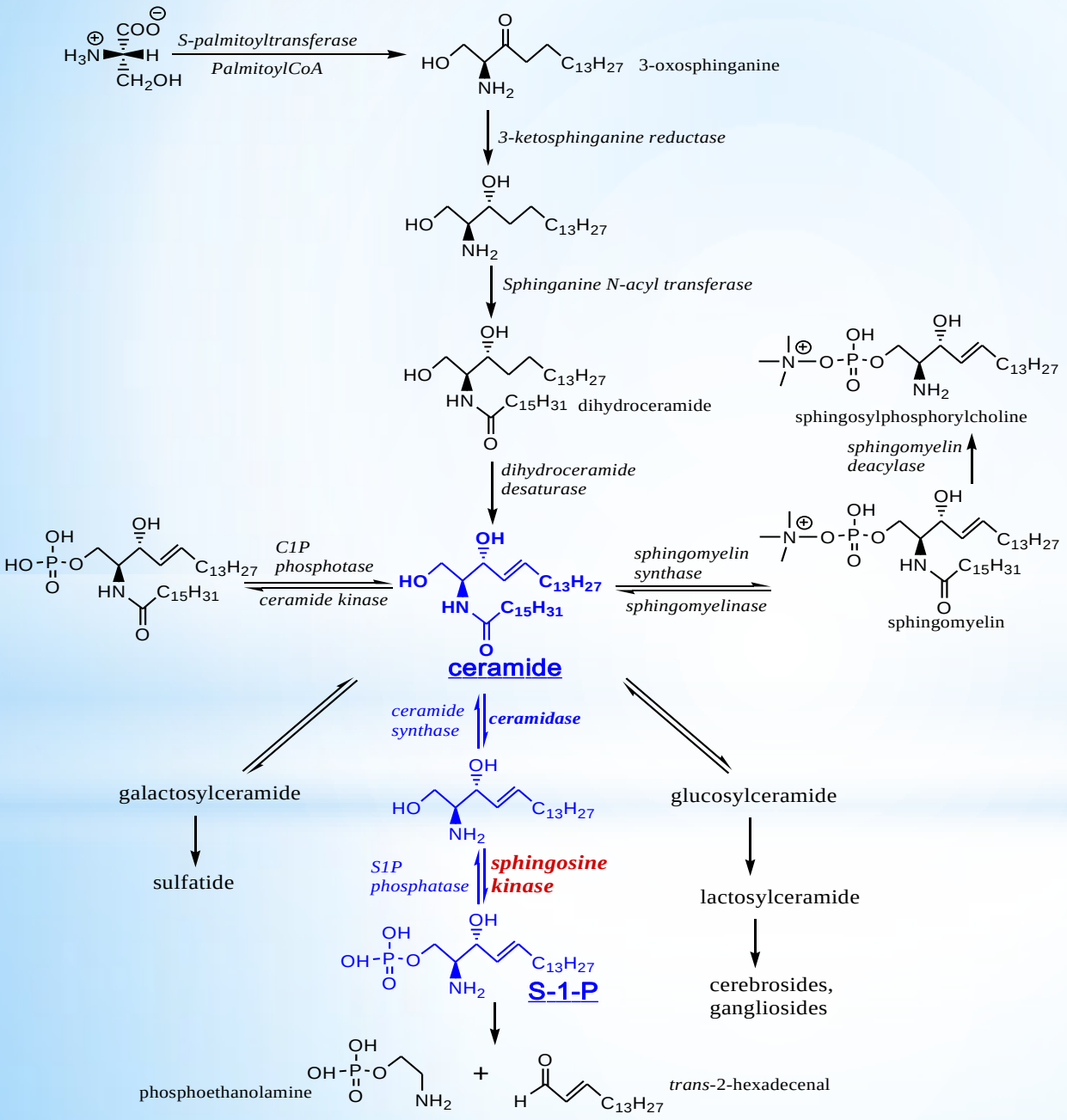
- **Sphingolipids, similar to phospholipids, are composed of a polar head group and two nonpolar tails. The core of sphingolipids is the sphingosine.**
- **Sphingolipids are one of the lipids forming the plasma bilayer membrane. They play important roles in cell signal transmission and recognition.**





X = H, Ceramide
= Phosphate, Ceramide-1-phosphate
= Glucose, Glucosylceramide

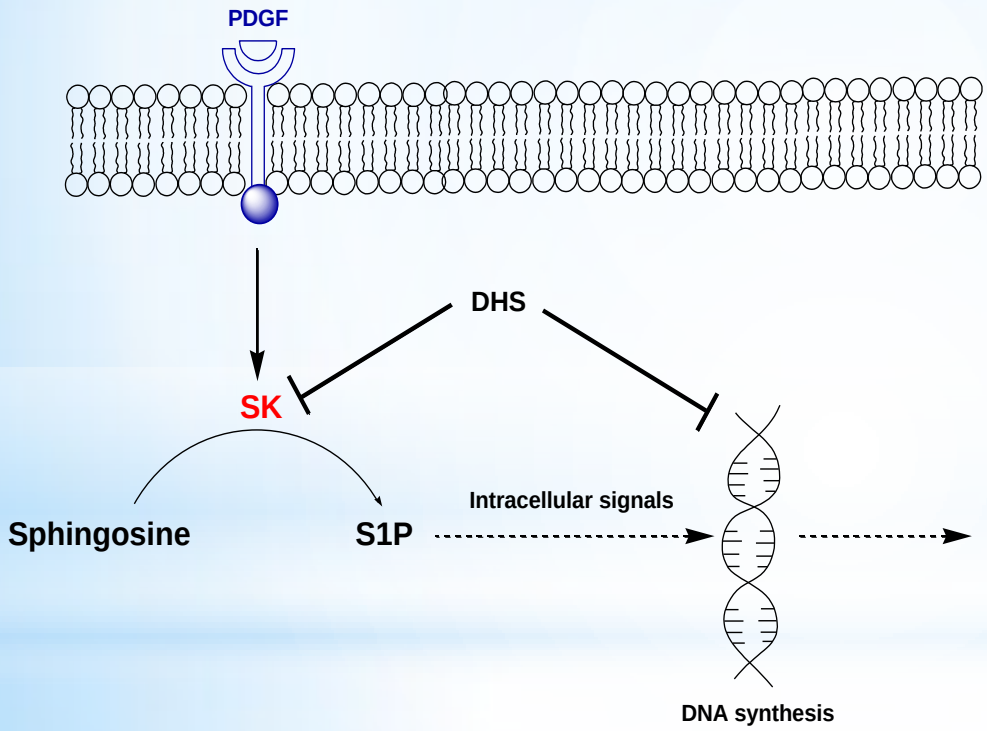
Sphingolipid Anabolism and Catabolism



- Sphingosine Kinase (SK) is one of the critical enzymes in sphingolipid biosynthesis.
- SK catalyzes sphingosine phosphorylation.
- There are two isoforms of sphingosine kinase (SK): SK1 and SK2.
- The convincing evidences have shown that SK, especially SK1, connects with human diseases, such as cancer.

Validation of SK as a druggable target for cancer therapy

1. Inhibition of SK lowered the DNA synthesis in NIH3T3 cells.



1.GF activated SK in 3T3 cell, which stimulated the production of S1P.

2.DHS not only inhibited SK and reduced S1P but also lowered DNA synthesis in the cell.

3.S1P is a second messenger to promote the cell proliferation.

Nature, 1993, 365:557

2. SK1 plays an oncogenic role in NIH3T3 cells.

SK1-transfected 3T3 cells acquired the tumorigenic phenotype:

(1) To proliferate in serum-free medium.

(2) To form fibrosarcomas when transplanted into nude mice.

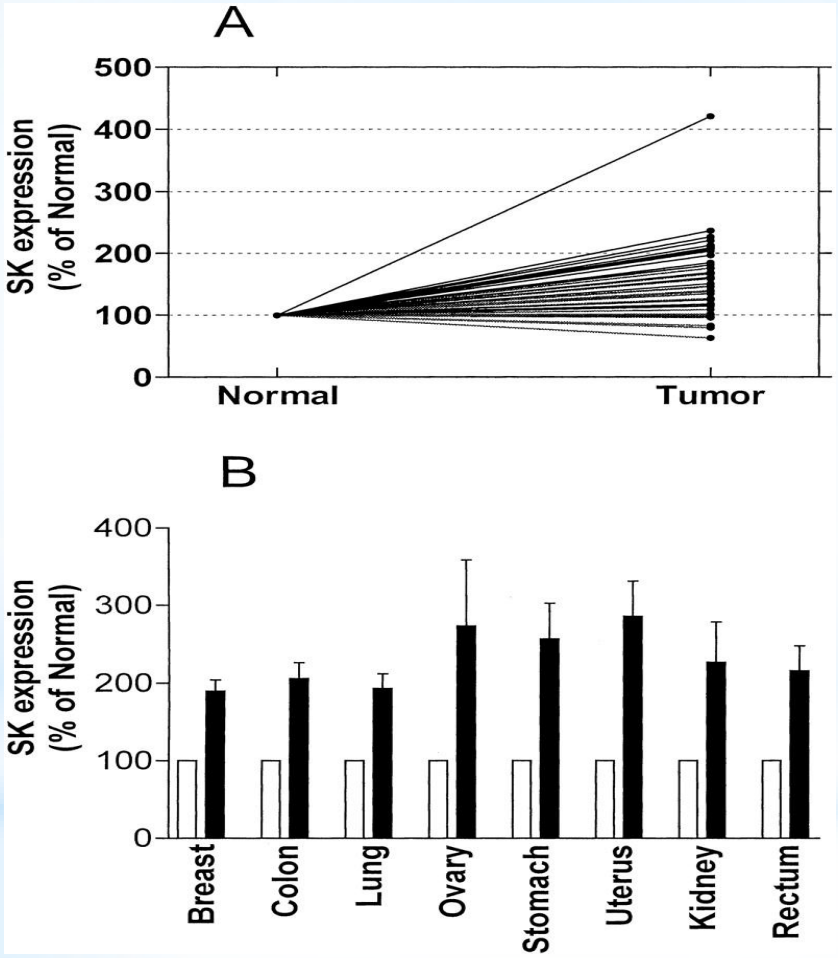
(3) The above effects were absent for 3T3 cells expressing inactive SK1 (SK1G82D mutant) or SK1-transfected 3T3 cells treated with DMS, a non-specific SKI.

Current Biology 2000, 10(23), 1527

3. SK1 KO Mice displayed no abnormalities.

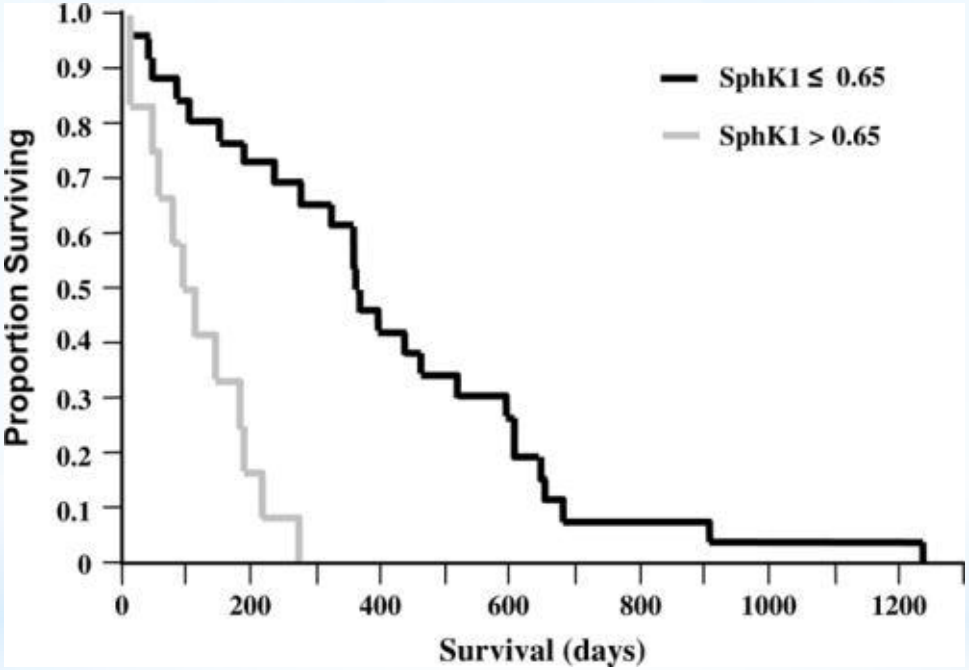
J. Biol. Chem. 2004, 279, 52487

3. SK mRNA was significantly higher in tumor tissues than in the adjacent normal tissues



Cancer Res 2003, 63:5962

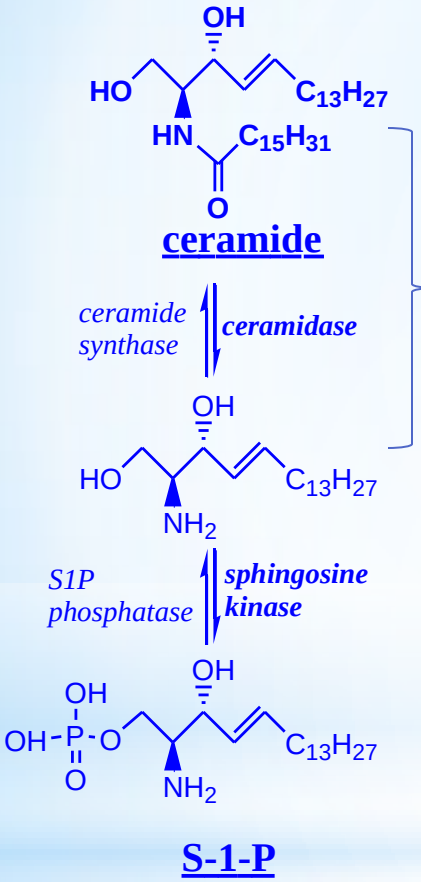
4. SK1 expression was related to the survival of the patients with glioblastoma multiforme (GBM).



GBM patients with low levels of SK1 expression had a median survival of 357 days.
GBM patients with high levels of SK1 expression had a median survival of 102 days.

J Neuropathol Exp Neurol, 2005, 64(8), 695

5. SK1 overexpression related to the resistance of anticancer treatments.

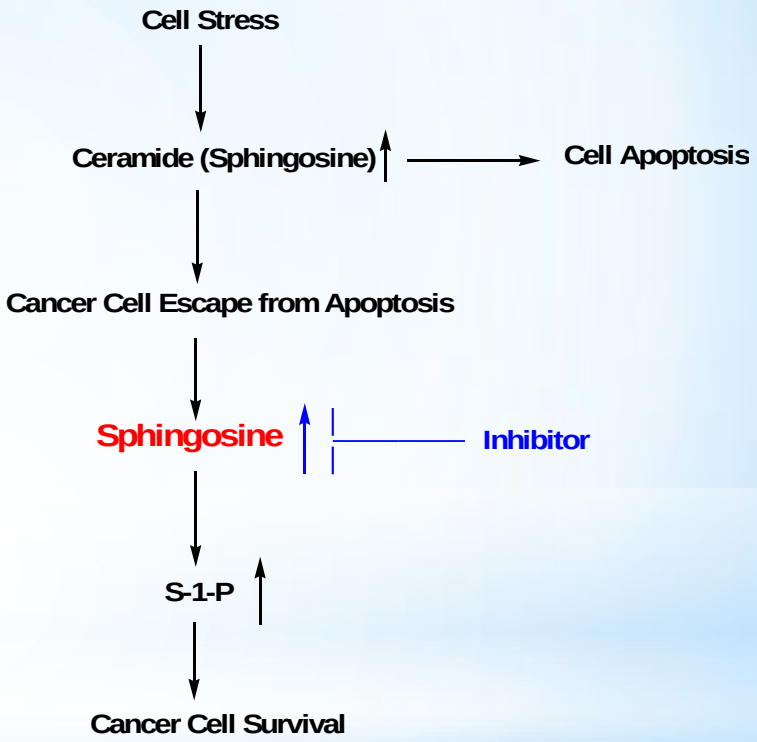


Ceramide/S1P Biostat

Pro-apoptotic

Anti-apoptosis,

Angiogenisis



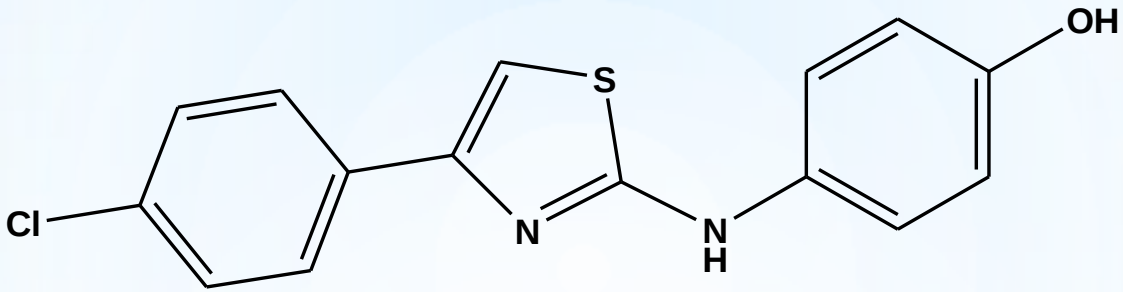
SK1 overexpression could markedly inhibit cell death induced by (i) anthracyclines in MCF7 cells (*Exp Cell Res 2002, 281,115*); (ii) doxorubincin and etoposide in HL60 cells (*Leukemia 2006, 20, 95*); (iii) camptothecin and docetaxel in PC3 and LNCaP cells (*Cancer Res 2005, 65, 11667*).

High-throughput Screening (HTS) and Hit Identification

We did small molecule HTS and a few of hits were identified.

Cancer Res 2003, 63:5962

SKI-2 (*4-[4-(4-Chlorophenyl)thiazol-2-ylamino]phenol*)



1. **Potent inhibitor**
2. **Selective inhibitor**

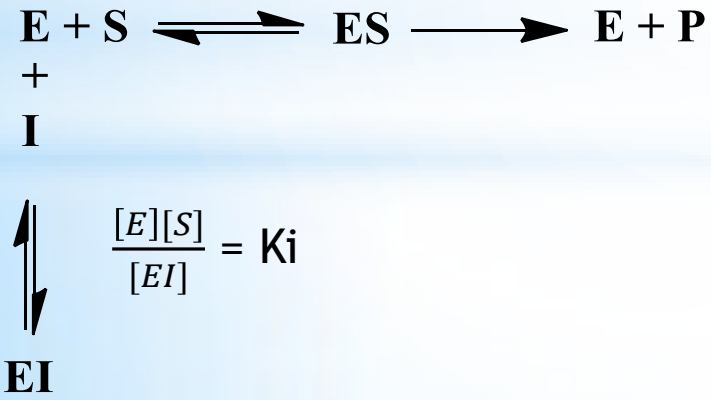
3. **Lipinski's Rule**
   Molecular Weight: 302.78
   CLogP: 4.25604

Compound	SK (IC50 μM)	ERK2	PI3K	PKC
SKI-2	0.5 ± 0.3	None	None	None

J Pharmacol Exp Ther 2006, 318:596

SKI-2 Binding Constant Ki

Enzyme	Ki of SKI-2 (μM)	Ki of DMS (μM)
SK1	0.69 ± 0.24	52 ± 10
SK2	0.13 ± 0.02	22 ± 9



The binding constant Ki is a parameter to measure the potency of an enzyme inhibitor.

Unpublished Data

1. SKI-2 against SK1 in Chronic Myeloid Leulemia

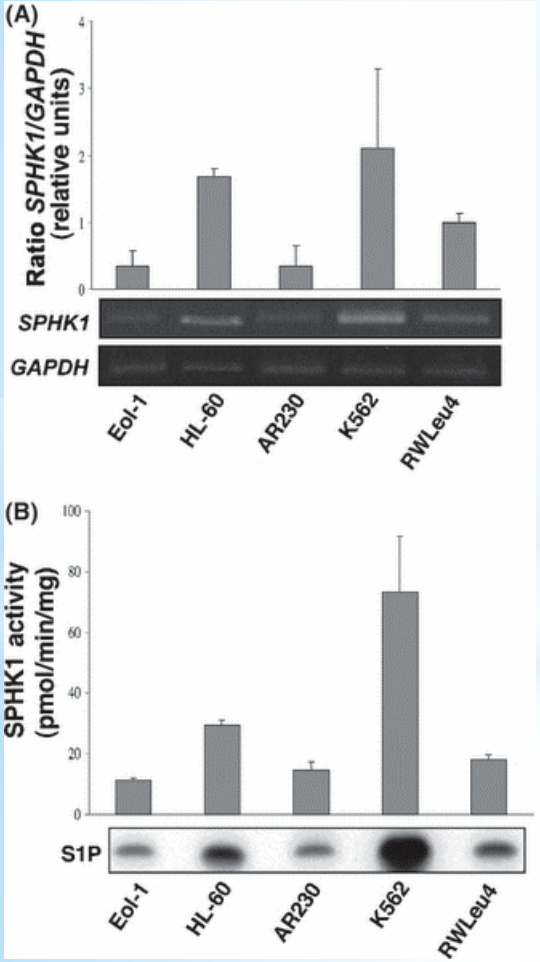


Fig 1. SK1 expression and activity in different myeloid leukaemia cell lines. (A) SK1 expression; (B) The S1P is correlated with SK1 activity.

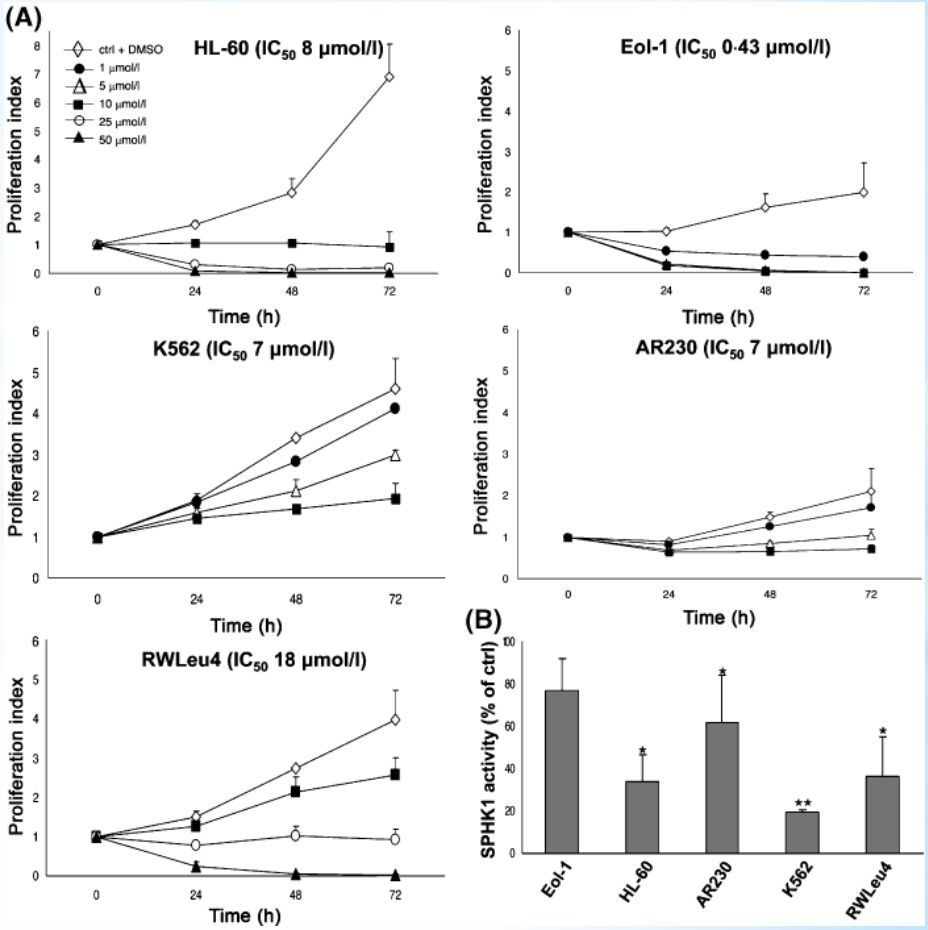


Fig 2. Effect of SKI-2 against SK1on the survival of CML cells. (A) cells were treated with SKI-2; (B) SK1 activity was after exposure to SKI-2 at 48 h.

There was a correlation between the cell proliferation and SK1 activity.

In Vitro Combination Therapy Using SKI-2 with Imatinib

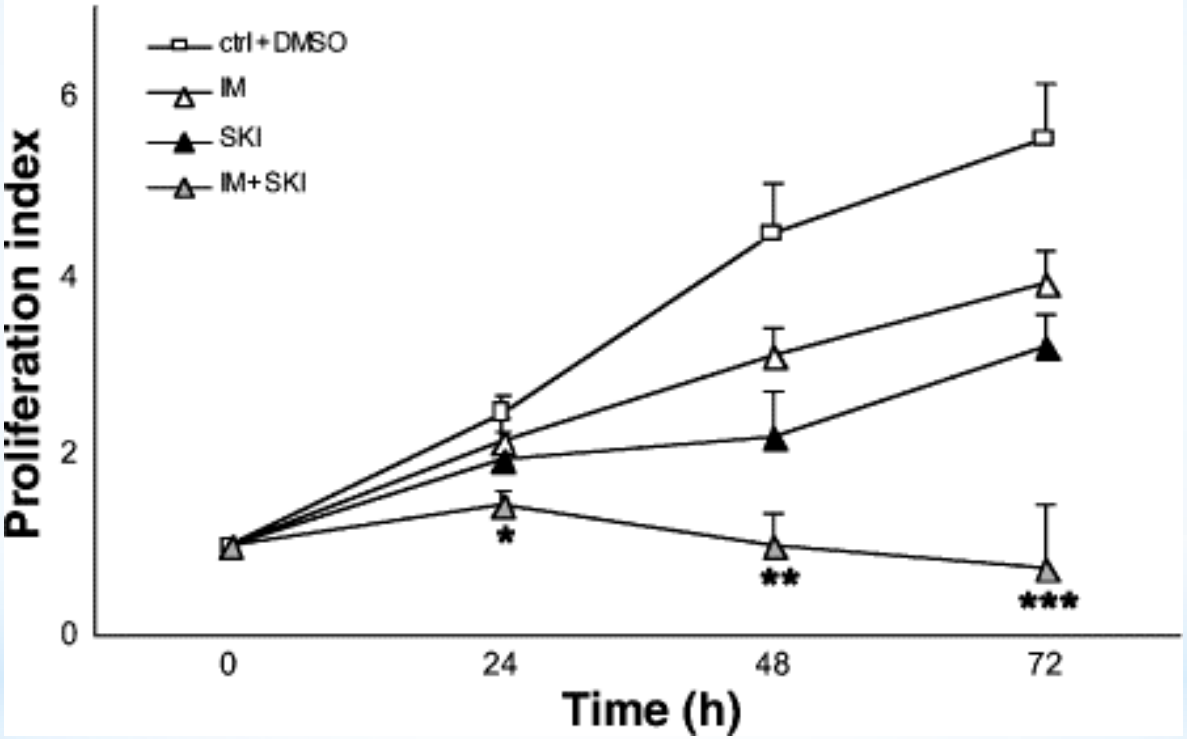


Fig 3. Effect of the simultaneous exposure of K562 cells to SKI-2 and IM (imatinib mesylate). Either alone or in combination. There was a synergistic effect of the combination therapy. CI = 0.061 calculated at 24 h

BJH 2008, 144, 350

2. SKI-2 aganist TMZ resistant glioblastoma

Mol Cancer Ther 2009;8(4):809

The temozolomide (TMZ) has shown to provide a survival benefit for Glioblastoma patients. While the initial treatment of many GBM with TMZ shows dramatic results, virtually all tumors recur and become resistant to TMZ.

An increase of SK1 gene expression has been observed to the TMZ-resistant GBM cells. SKI-2 was very effective in the treatment of the TMZ-resistant glioblastoma cells.

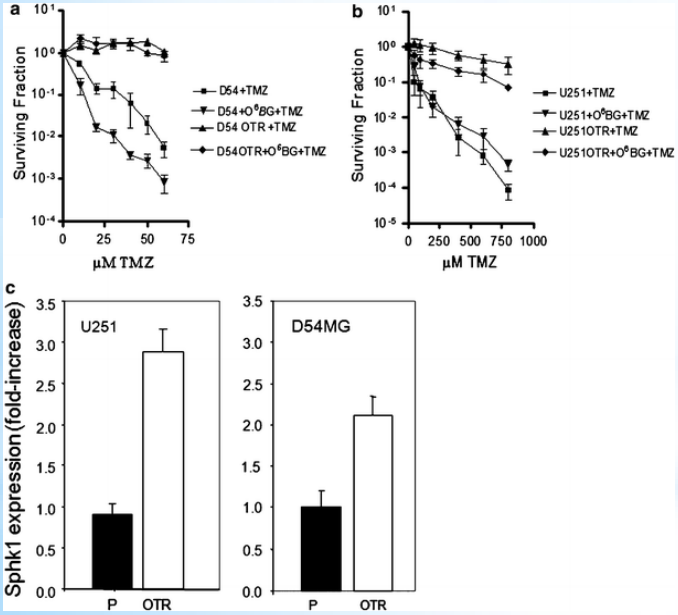


Fig. 1 Cells with TMZ resistances were correlated with the increase of SK1 expression.

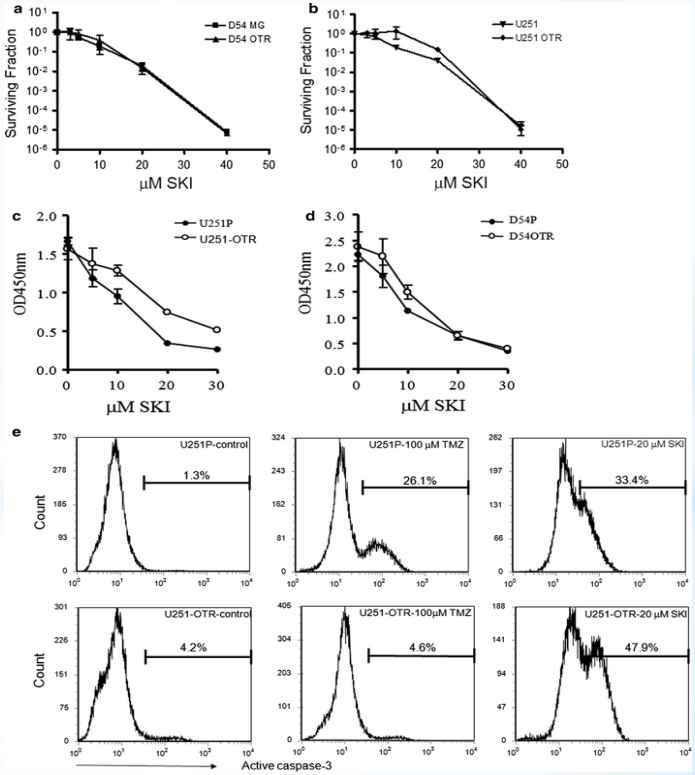


Fig. 2. Effect of SKI-2 on cell proliferation were shown on both sensitive and resistant to TMZ cells.

e Caspase-3 activation in U251 cells upon SKI treatment was measured using flow cytometry.

SKI-2 drawbacks

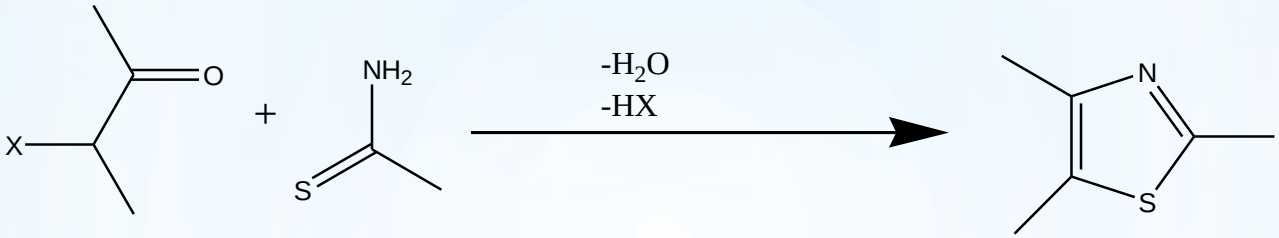
(1) Low bioavailable (*J Pharmacol Exp Ther 2006, 318, 596*)

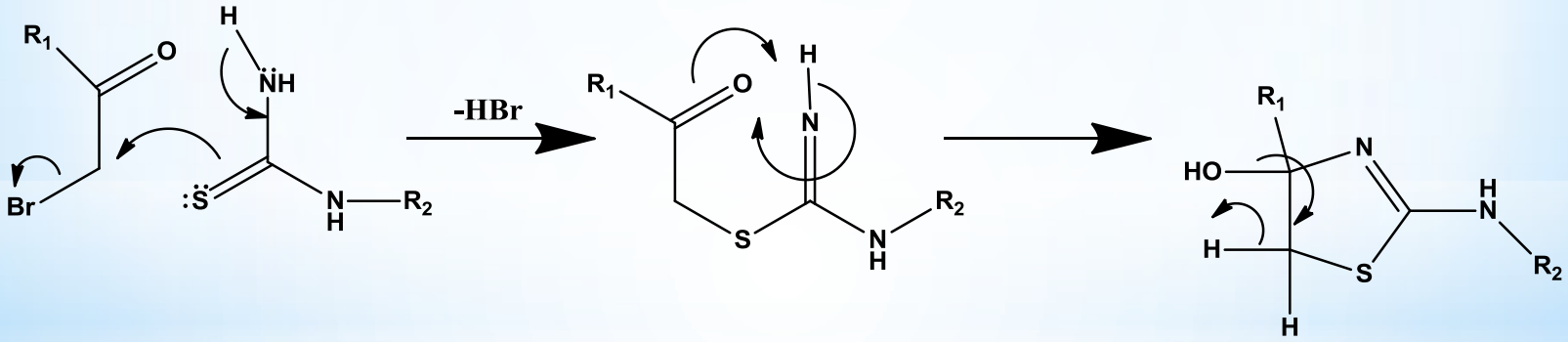
(2) In vivo toxic and unstable (*unpublished data*)

(3) Non-selective between SK1 and SK2

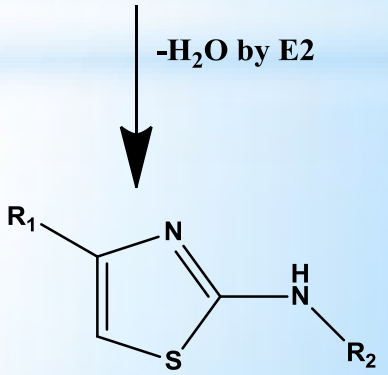
SKI-2 Optimization

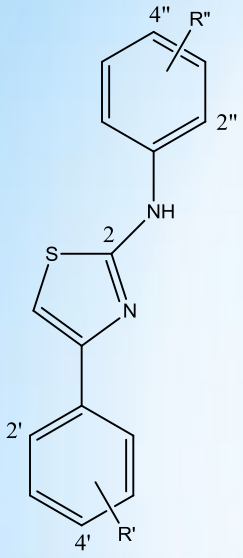
1. Synthesis of analogs: Hantzsch Thiazole Synthesis





The reaction goes by the way of an SN2 reaction. It begins an attack at α-carbon bearing the halogen by sulfur rather than nitrogen to form an intermediate, which was then undergoing ring formation, followed by E2 elimination to remove a molecule of water.





SAR Analysis

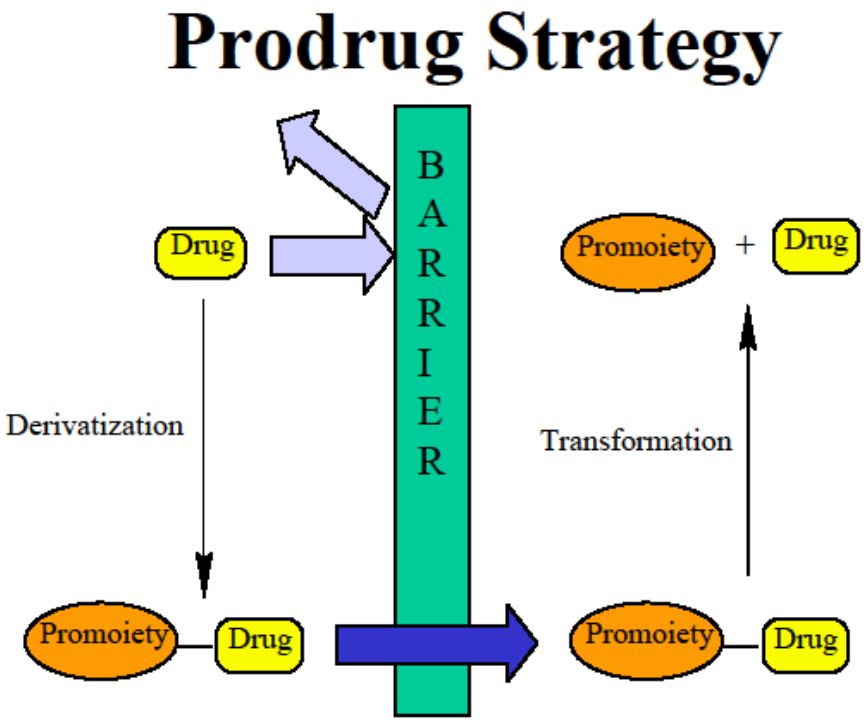
• 4”- or 2”-OH is essential to inhibit SK1.

• Substitution on ‘- ring has no significant influence for SK inhibition.

• Nearly all SKI-2 analogs are good SK2 inhibitors

Compound	5-C	R’2	R’4	R’6	R”2	R”3	R”4	SK1 IC50	SK2 IC50
BB090420	Ph	H	H	H	H	H	CH3	>100	1.8
BB090421	Ph	H	H	H	H	H	OH	3.7	<0.8
BB090422	Ph	H	CF3	H	H	H	OC2H5	2.3	<0.8
BB090430	Ph	H	F	H	H	H	OH	1.3	<0.8
BB090501	Ph	H	F	H	H	H	H	>100	<0.8
BB090528	Ph	H	CH3	H	H	H	OH	1.5	<0.8
BB090529	2-Na				H	H	OH	6.5	<0.8
BB090601	Ph	H	CH3	H	H	H	CH3	>100	<0.8
BB090602	2-Na				H	H	CH3	>100	15
BB090603	Ph	H	CH3	H	H	H	OCH3	>100	<0.8
BB090604	2-Na				H	H	OCH3	>100	3.3
BB090608	Ph	H	CH3	H	H	H	Cl	>100	3
BB090609	2-Na				H	H	Cl	>100	8.2
BB090610	Ph	H	CH3	H	H	H	CN	>100	1
BB090611	2-Na				H	H	CN	>100	2.3
BB090615	Ph	H	CH3	H	H	H	COCH3	>100	2.7
BB090616	2-Na				H	H	COCH3	>100	1.4
BB090617	Ph	OH	H	H	H	H	OH	1.2	<0.8
BB090618	Ph	OH	OH	H	H	H	OH	4.4	1
BB090622	Ph	H	H	H	OH	H	H	4.5	<0.8
BB090623	Ph	H	H	H	H	OH	H	15	1.5
BB090625	Ph	H	CH3	H	H	H	NO2	>100	6.5
BB090626	Ph	OH	OH	H	H	H	NO2	21	5
BB090629	Ph	H	NO2	H	H	H	OH	1.2	<0.8
BB090630	Ph	H	NO2	H	H	H	NO2	>100	<0.8
BB090701	Ph	OH	H	F	H	OH	H	23	<0.8
BB090707	Ph	OH	H	H	OH	H	H	3	<0.8
BB090713	Ph	NO2	H	H	OH	H	H	1.9	<0.8
BB090714	Ph	NO2	H	H	H	H	OH	0.9	<0.8
BB090716	Ph	NO2	H	H	H	OH	H	8	<0.8
BB090722	Ph	OH	H	H	H	H	OCH3	62.5	<0.8
BB090723	Ph	OH	OH	H	H	H	OCH3	75	<0.8
BB090727	Ph	OH	H	H	H	H	COOH	>100	<0.8
BB090728	Ph	OH	OH	H	H	H	COOH	78	<0.8
BB090825	Ph	NH2	H	H	H	H	OH	<0.8	<0.8
BB090831	Ph	H	Cl	H	H	H	OH	0.9	<0.8
BB090702	Ph	OH	H	F	H	H	OH	<0.8	<0.8
BB090708	Ph	OH	H	F	OH	H	H	2.5	<0.8
BB090504	Ph	H	F	H	H	H	CH3	>100	4.7

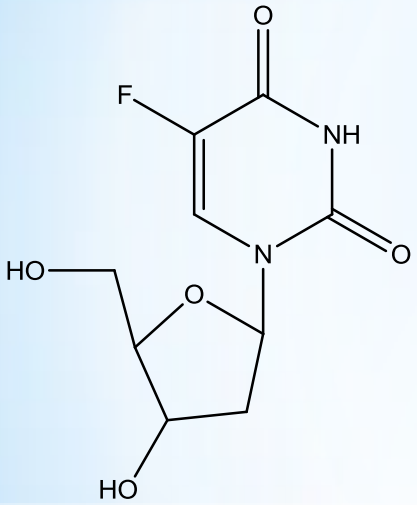
2. Prodrug Approach



Reference: Prodrugs: Challenges and Rewards (V. Stella, R. T. Borchardt, M. Hageman, R. Oliyai, J. Tilley and H. Maag, Eds Springer, New York, NY, 2007).

The prodrug: a covalent link between a drug and a chemical promoiety which are chemically or enzymatically labile in vivo. It is designed to circumvent the pharmaceutical or PK barriers of the parent drug, such as low bioavailability, toxicity and stability.

EX. to use Ester-prodrug to improve PK and therapeutic effect.



FDU: (1) a very potent anticancer drug *in vitro.*

(2) having been used in the clinic, such as colon cancer, pancreatic cancer, liver cancer, breast cancer and metastatic renal cancer.

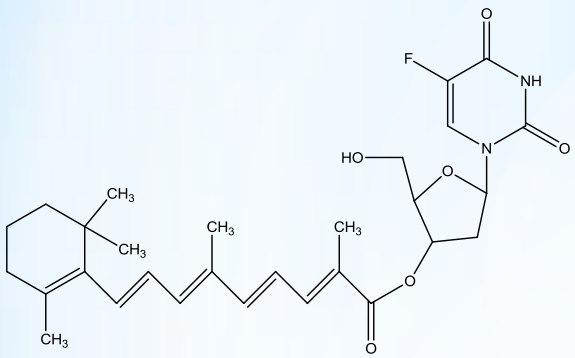
(3) Due to its poor bioavailability (too hydrophilic), FDU is not suitable for PO administration.

(4) Owing to its rapid elimination ($T_{1/2}$ = 7 min), FDU is most administered by intra-arterial infusion.

(5)There are other problems for FDU, such as metabolic unstable.

To make ester prodrug of FDU, we anticipated that the ester prodrug may act as a depot to release the active drug in vivo to improve the PK of the parent drug.

3'-*O*-RFDU (17) showed good properties.



- It had no acute or chronic toxicity to the mice under the therapeutic doses.
- (Fig 3-7-2) It was hydrolyzed in vivo to produce the parent drugs.
- (Fig 3-7-3) The PK was improved. The bioavailability was 90%.
- It showed higher anticancer potency than that of the parent drug used alone both in vitro and in vivo.

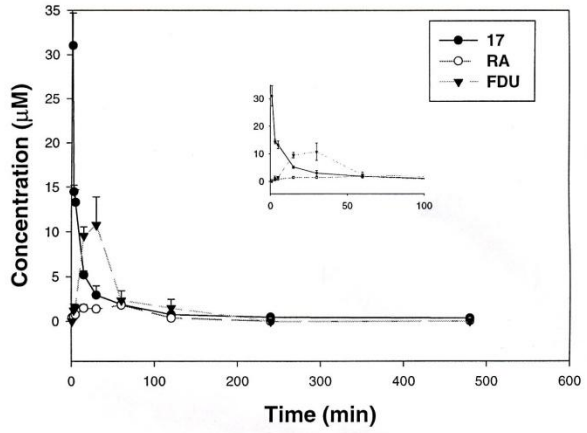


Figure 3-7-2. 3'-*O*-RFDU (**17**), RA and FDU concentration-time profiles in mouse plasma after a single i.v. bolus injection of (**17**) (12.5 μmol/kg). Error bars represent SD (n = 3).

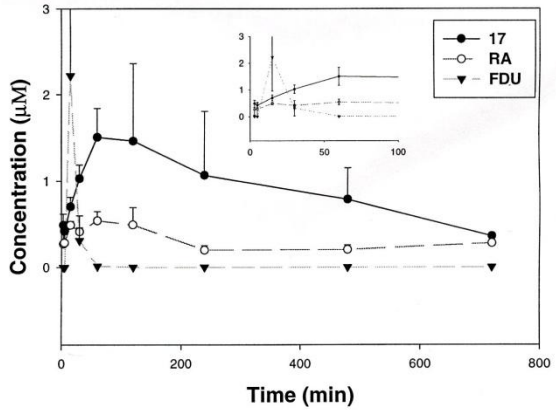
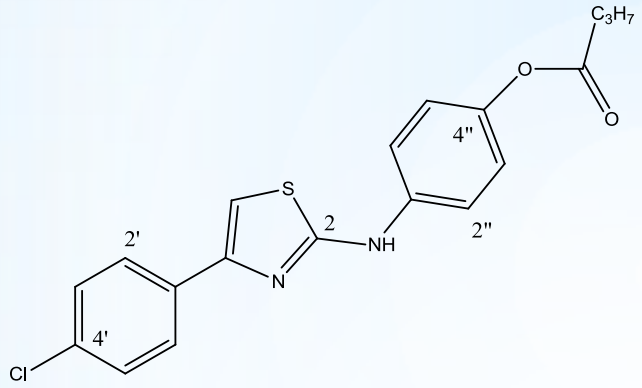


Figure 3-7-3. 3'-*O*-RFDU (**17**), RA and FDU concentration-time profiles in mouse plasma after a single p.o. dose of (**17**) (13.7 μmol/kg). Error bars represent SD (n = 3).

SKI-2 Ester Prodrugs

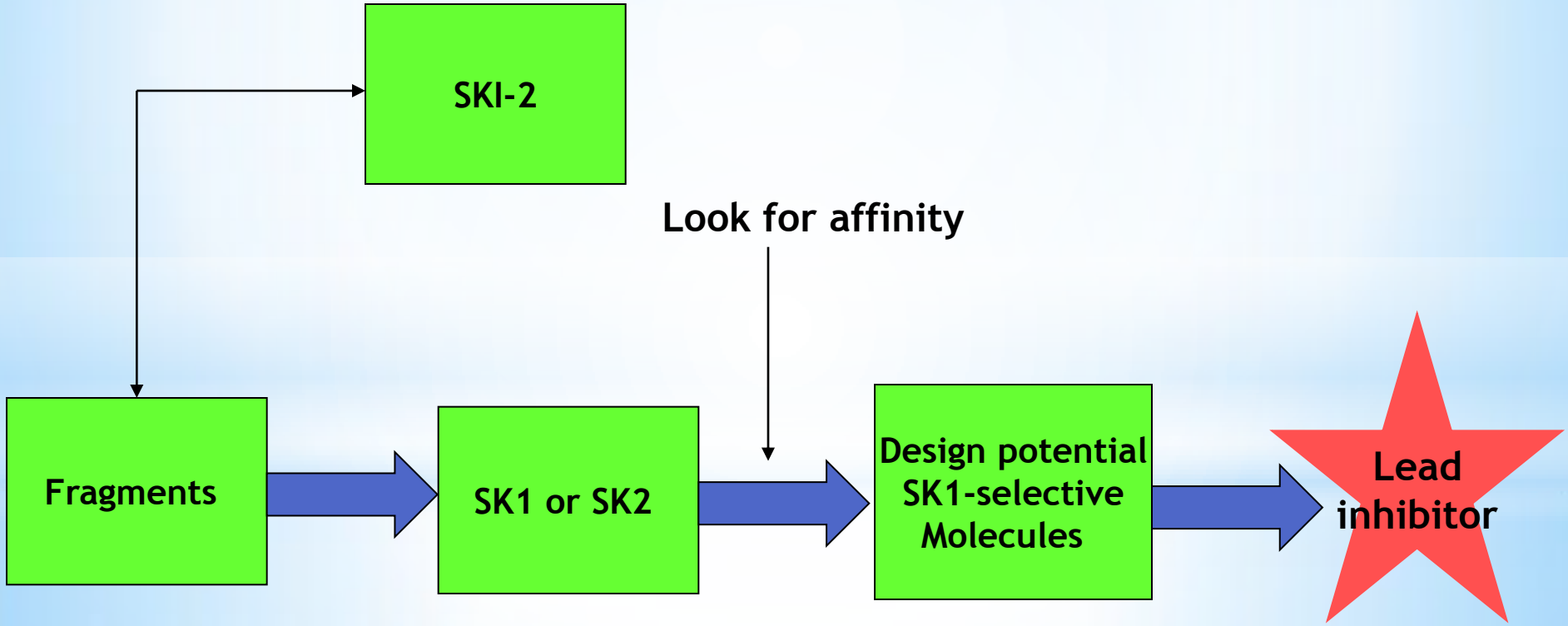


- The prodrugs are the prodrug only for SK1. They are potent inhibitors for SK2.
- The prodrug development is a iterative process.

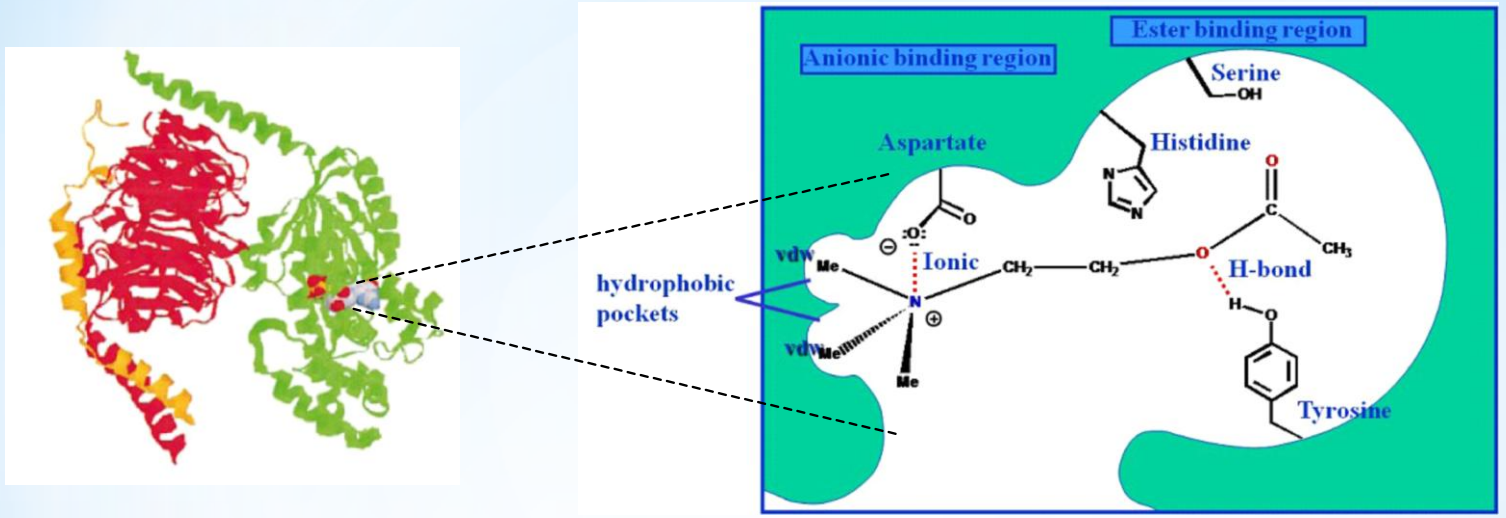
Compound	5-C	R'2	R'4	R'6	2-N	R"2	R"3	R"4	SK1 IC50	SK2 IC50
BB090908	Ph	H	Cl	H	OCOCH3	H	H	OCOCH3	>100	<0.8
BB090909-1	Ph	H	Cl	H	H	H	H	OCOC3H7	>100	<0.8
BB090909-2	Ph	H	Cl	H	OCOC3H7	H	H	OCOC3H7	>100	<0.8
BB090917	Ph	H	Cl	H	H	H	H	OCOCH3	75	<0.8

3. Fragment-based drug design:

An approach that uses small and simple molecules (fragments) to look for SK-inhibitor pharmacophores that may assist to design SK-selective inhibitor.

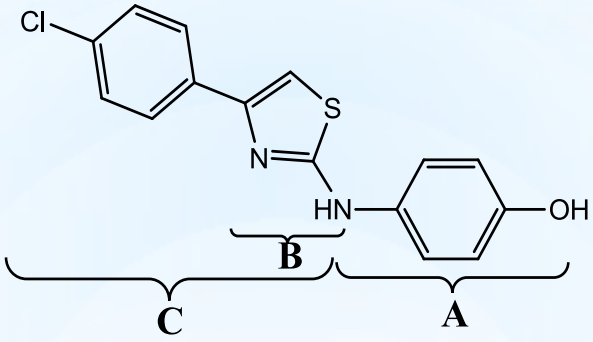


Pharmacophore

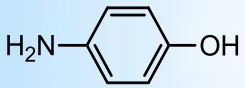


1. Enzymes are proteins. The active site is usually small and specific.
2. It may be only a small part of the SKI-2 that involves in the critical interaction with either SK1 or SK2. The relevant part on SKI-2 responsible for the activity of the inhibition is its pharmacophore.
3. The other parts in SKI-2 may be extraneous, even interfering with the binding of the pharmacophore.

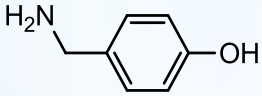
SKI-2



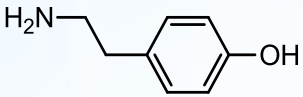
A1



A2

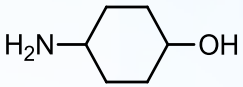


A3



tyramine

A4



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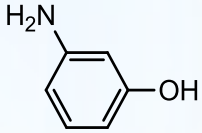

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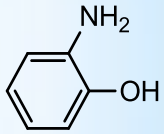
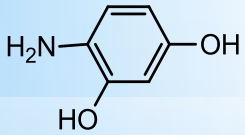
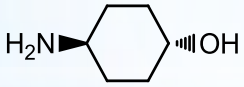


A7



4-aminoresorcinol

A8



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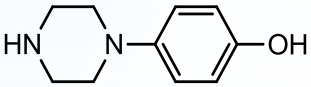

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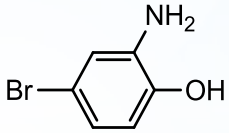

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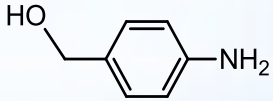
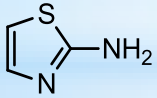
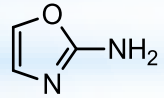


B1

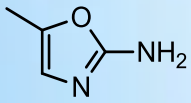


B2



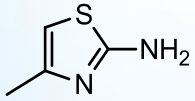
Oxazol-2-ylamine

C1



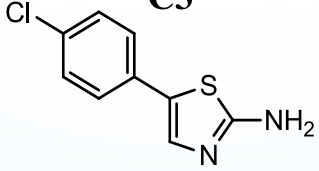
5-Methyl-oxazol-2-ylamine

C2

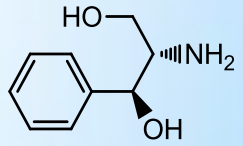


4-Methylthiazol-2-ylamine

C3

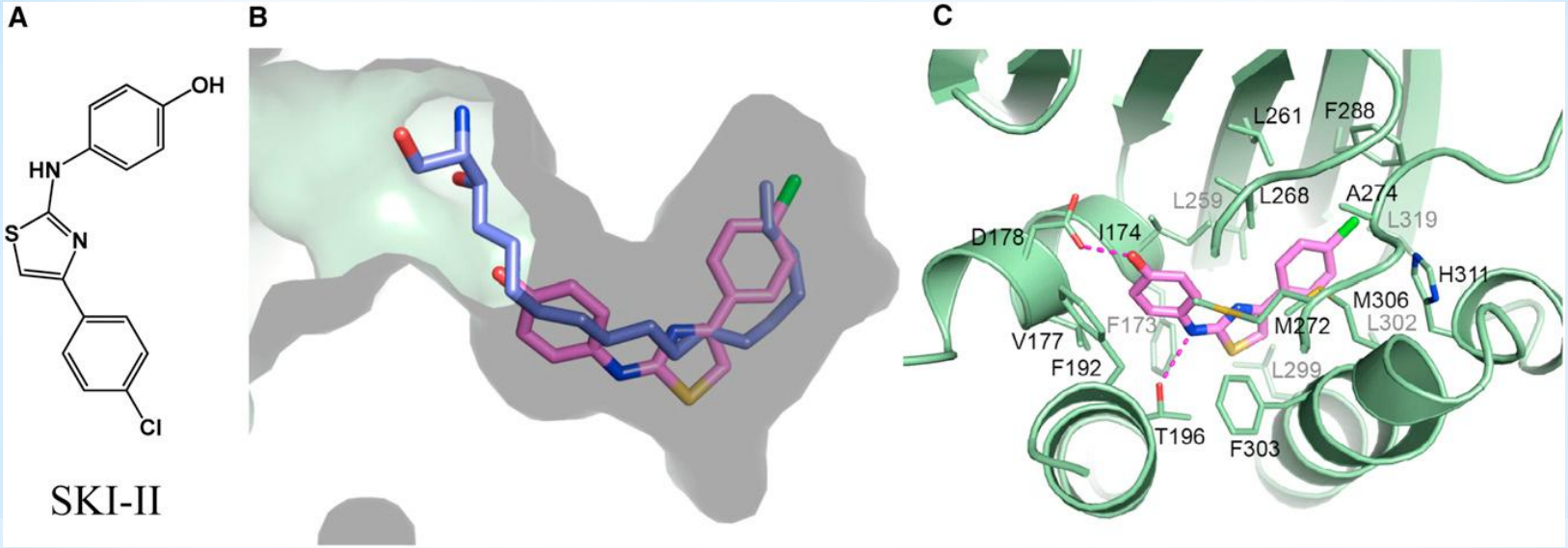


D1



(1S,2S)-2-amino-1-phenyl-1,3-propanediol

Crystal Structures of SK1, SK1-Sphingosine and SK1-SKI-2 have been resolved.



(A) Chemical structure of SKI-II.
(B) SKI-II binding in the lipid-binding pocket of SK1 with overlaid sphingosine.
(C) Detailed ligand-protein interactions between SKI-II and SK1.

These structures will lead to better understanding of the small molecules binding with SK1 and hopefully to develop better therapeutic molecules.

Structure 2014, 21, 798–809

Summary

1. We have found SK2-selective inhibitor.

2. Although we have not found SK1-selective inhibitor yet, we are getting closer.

3. We believe cancers have complicated signaling survival pathways. Currently, the most effective treatment for cancer commonly require target multiple pathways. So we will test and optimize the combination therapy using SK-selective inhibitors with other anticancer drugs or therapies.

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