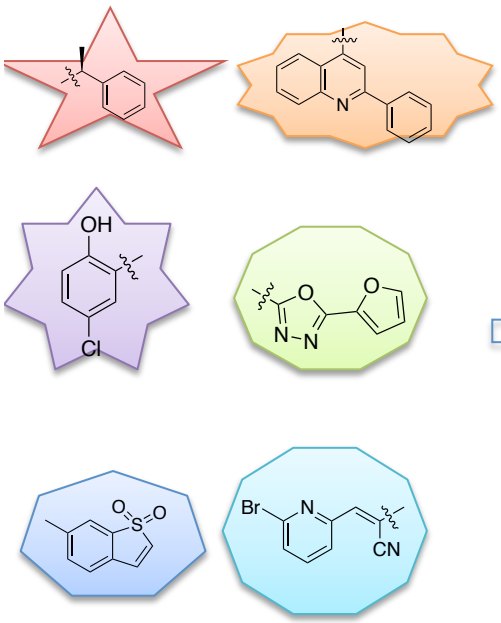
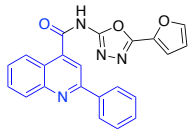
Fragment-based Drug Design and Identification of HJC0123, A Novel Orally Bioavailable STAT3 Inhibitor for Cancer Therapy

Chen, H.; Yang, Z.; Ding, C.; Chu, L.; Zhang, Y.; Terry, K.; Liu, H.; Shen, Q.; Zhou, J. TX, US

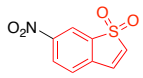
EJMC. 62 (2013) 498-507



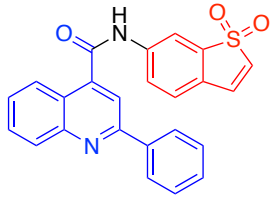
Fragment-Based Drug Design



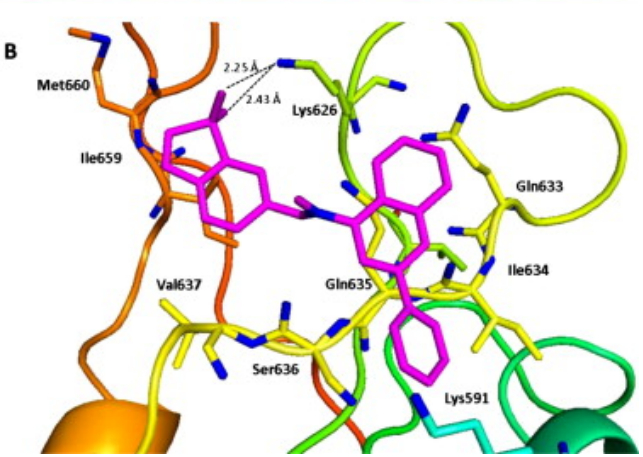
STX-0119
inhibit STAT3 dimerization



Stattic
non-peptidic STAT3 inhibitors
inhibit phosphorylation

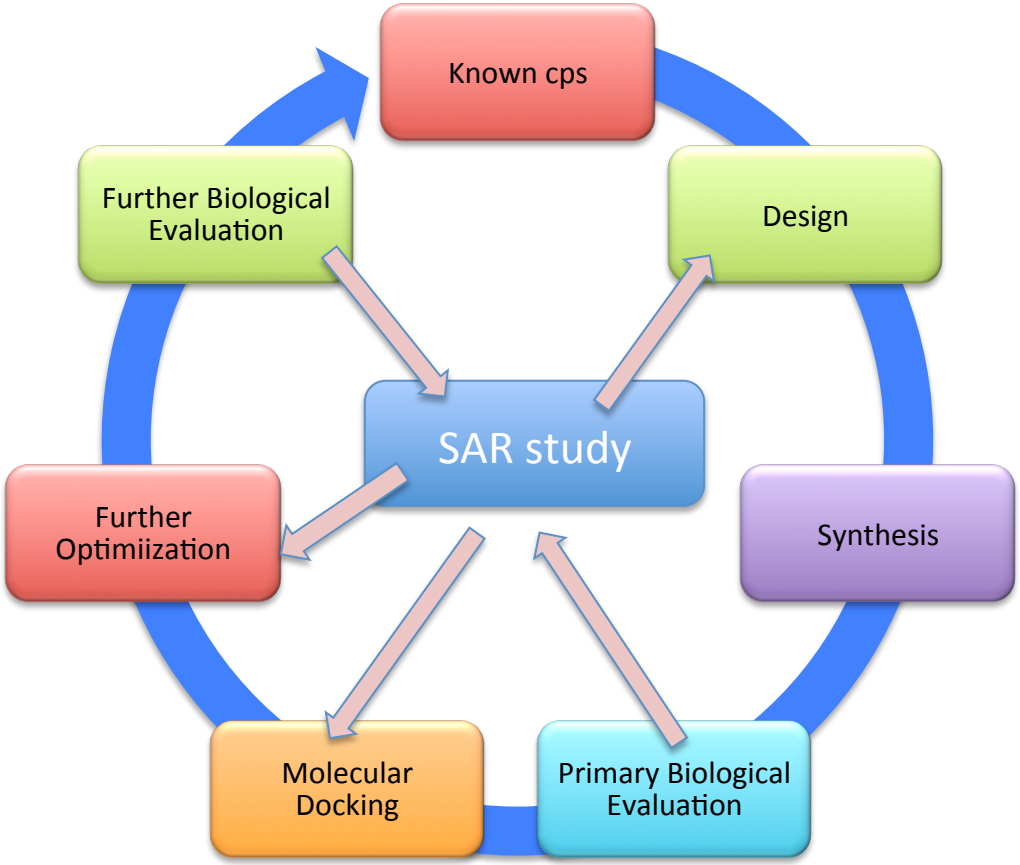


MCF-7: 0.1 μm
MDA-MN-231: 0.29 μm
Orally bioavailable



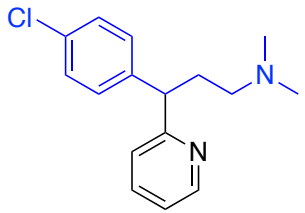
Fangfei Qin
Wipf Group Current Literature
May 11, 2013

Research Scheme



Let's Start with A Story…

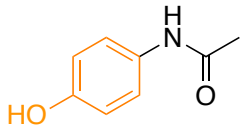
- In 2004, when I was 14 years old…



Chlorphenamine:
first-generation alkylamine antihistamine

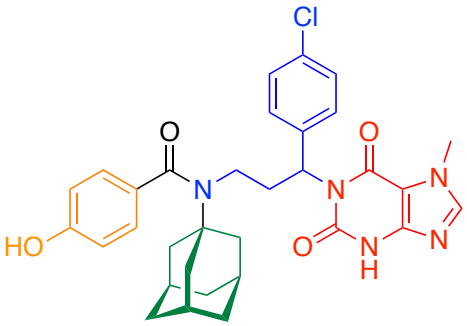
2 mg

Compound Paracetamol and Amantadine Hydrochloride Capsules

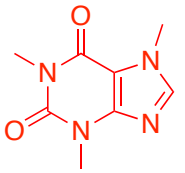


Paracetamol:
analgesic and antipyretic

250 mg



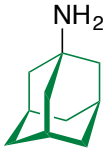
My imagination



Caffeine:
CNS stimulant

15 mg

Ingredients



Amantadine:
antiviral

100 mg

Fragment-Based Drug Design (FBDD)

Fortunately…

June, 2004

J. Med. Chem., **2004**, *47* (14), 3463–3482

1 month before

July, 2004

My birthday!

1 month Later

August, 2004

Nat. Rev. Drug Discov.**2004**, *3*(8), 660-672

Fragment-Based Drug Discovery

Daniel A. Erlanson ,* Robert S. McDowell ,* and Tom O'Brien *
Sunesis Pharmaceuticals, Inc., 341 Oyster Point Boulevard, South San Francisco, California 94080

J. Med. Chem., **2004**, *47* (14), pp 3463-3482
DOI: 10.1021/jm040031v

Fragment-based lead discovery.

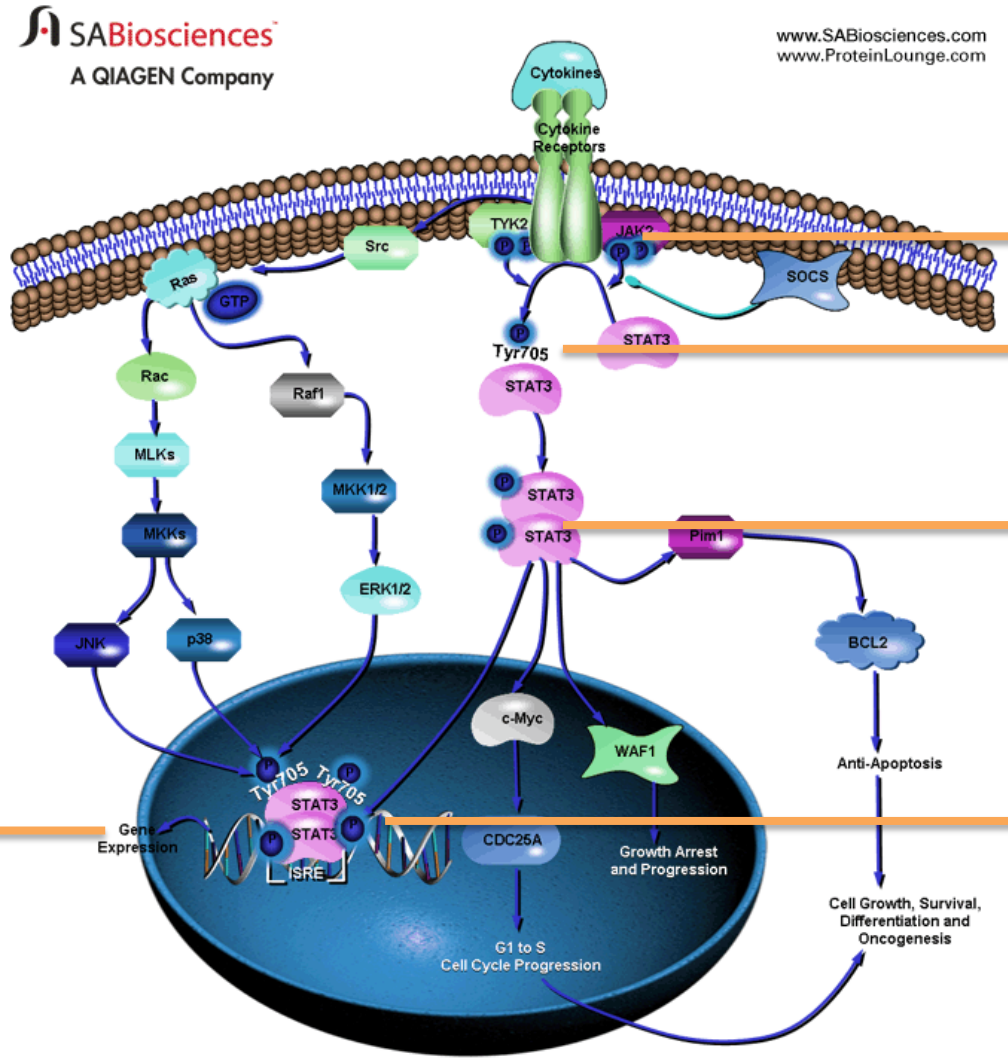
Authors: Rees, David C.[1] *d.rees@astex-technology.com*
Congreve, Miles[1]
Murray, Christopher W.[1]
Carr, Robin[1]

Source: Nature Reviews Drug Discovery. Aug2004, Vol. 3 Issue 8, p660-672. 13p.

Fragment-based drug design (FBDD) is a promising approach for the generation of lead molecules with enhanced activity and especially drug-like properties against therapeutic targets.

[1] D.A. Erlanson, R.S. McDowell, T. O'Brien, Fragment-based drug discovery, J. Med. Chem. 47 (2004) 3463-3482.
[2] D.C. Rees, M. Congreve, C.W. Murray, R. Carr, Fragment-based lead discovery, Nat. Rev. Drug Discov. 3 (2004) 660e672.
[3] P.J. Hajduk, Fragment-based drug design: how big is too big? J. Med. Chem. 49 (2006) 6972e6976.
[4] K. Babaoglu, B.K. Shoichet, Deconstructing fragment-based inhibitor discov- ery, Nat. Chem. Biol. 2 (2006) 720e723.

STAT3 Signaling Pathway VS Cancer Therapy



Inhibit JAK:
WP1066, AG490

Inhibit phosphorylation:
Stattic

Inhibit STAT3 dimerization:
PY*LKTK, STX-0119, S31-201

Inhibit DNA binding:
CAP-1, IS3 295

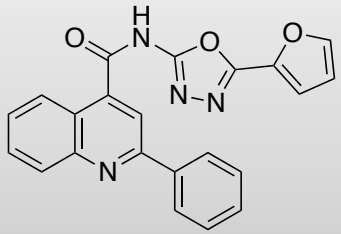
Inhibit transcription:
niclosamide

STAT3 Inhibitors

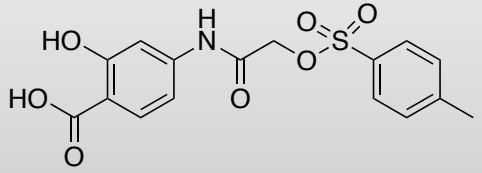
Inhibit STAT3 dimerization

H-Pro-Tyr-(PO_3H_2)-Leu-Lys-Thr-Lys-Ala-Ala-Val-Leu-Leu-Pro-Val-Leu-Leu-Ala-Ala-Pro-OH. CF3CO2H

PY*LKTK

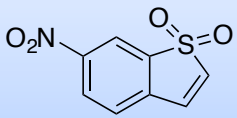


STX-0119



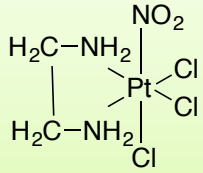
S31-201

Inhibit phosphorylation

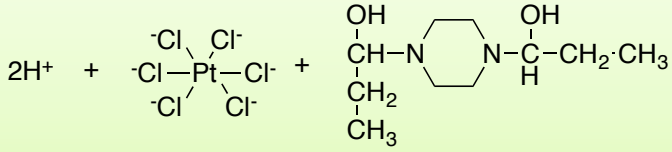


Stattic

Inhibit DNA- binding

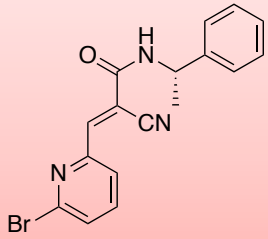


CPA-1

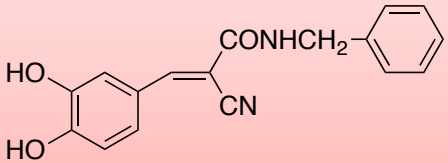


IS3 295

Inhibit transcriptional function of STAT 3



WP1066



AG490

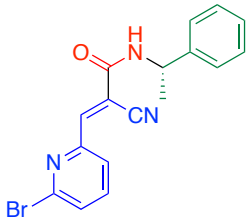
Me too, Me better!

JAK

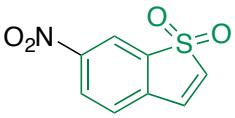
phosphorylation

dimerization

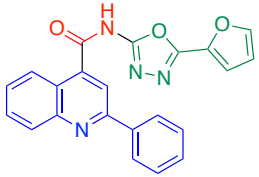
transcription



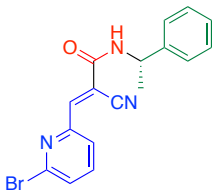
WP1066



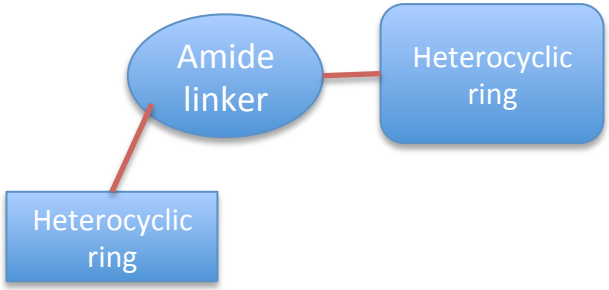
Stattic

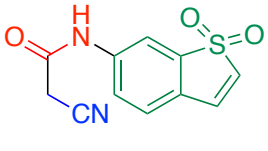


STX-0119



WP1066





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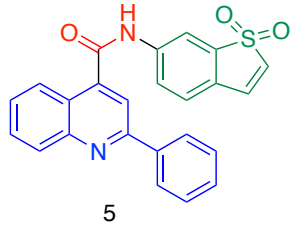

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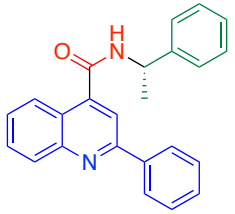

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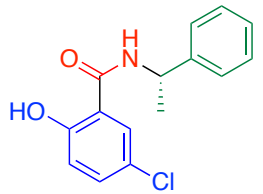

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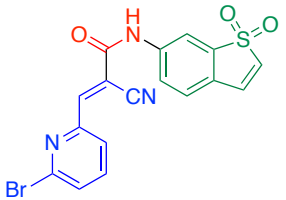

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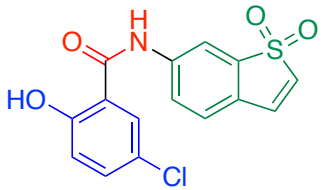
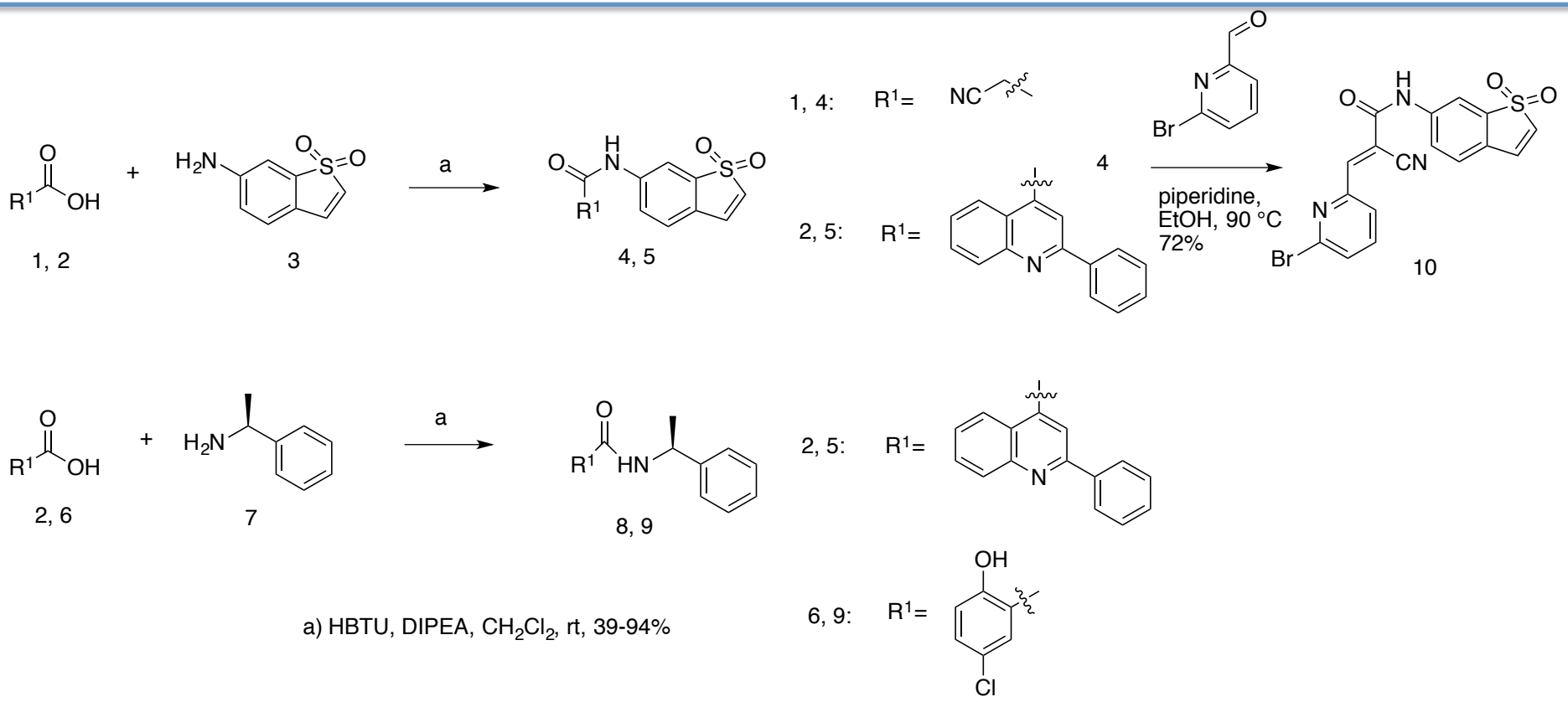


12

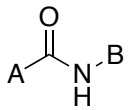
MW=120~251

Synthetic Route

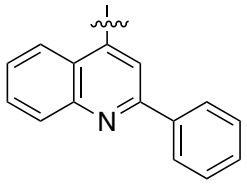


(b) $SOCl_2$, toluene, reflux; (c) R^2NH_2, pyridine, DMF, 0 °C to rt; (d) 1 N LiOH (aq.), THF, H_2O, 0 °C to rt, 39-50% (three steps).

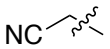
Biological Evaluation—SAR Study



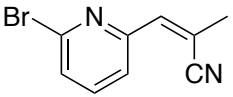
A =



A1



A2



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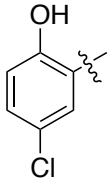
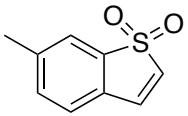
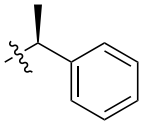


A4

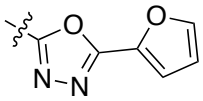
B =



B1



B2



B3

Compound	A	B	IC_{50} (μM)[a]			
			Breast cancer ER-Positive	Breast cancer ER-Negative	Pancreatic cancer	
			MCF-7	MDA-MB-231	AsPC1	Panc-1
4	A2	B1	>10[b]	>10	ND[c]	ND
5	A1	B1	0.1	0.29	1.25	0.26
8	A1	B2	2.24	86.0	>10	>10
9	A4	B2	0.9	8.88	7.54	8.44
10	A3	B1	3.31	1.53	1.54	1.64
12	A4	B1	0.91	1.64	1.92	2.34
13	A4	B3	>10	>10	>10	>10
Niclosamide			1.06	0.79	1.47	1.73

A1>A2

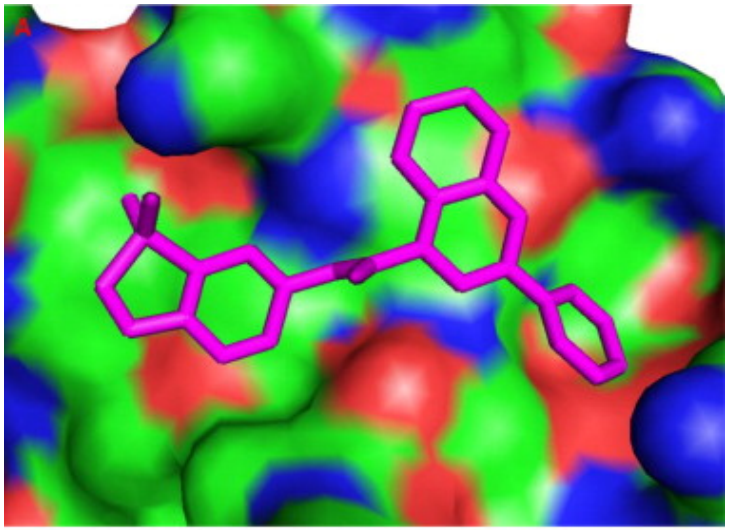
A1>A4

B1>B3

[a] Breast cancer cell lines: MCF-7 and MDA-MB-231. Pancreatic cancer cell lines: ASPC1 and Panc-1. Software: MasterPlex ReaderFit 2010, MiraiBio, Inc.
[b] If a specific compound is given a value >10, indicates that a specific IC_{50} cannot be calculated from the data points collected, meaning 'no effect'.
[c] ND: not determined.

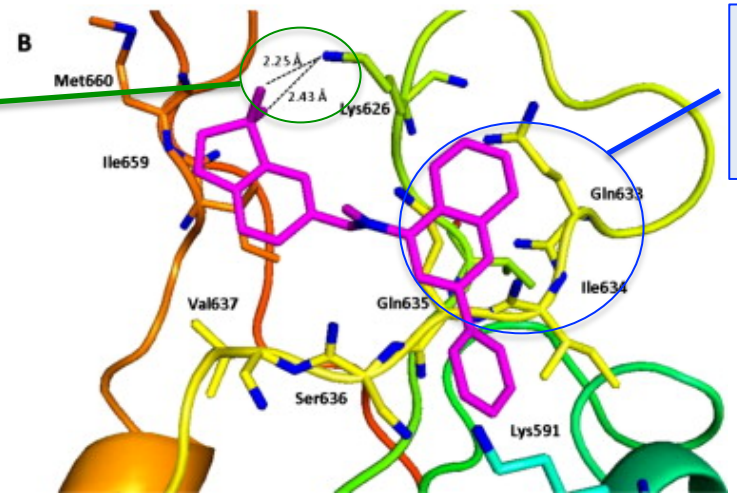
Molecular Docking Studies

A1, B1:
STAT3-SH2 domain



Surface of the electrostatic map.

Hydrogen bond



Quinoline ring could fit effectively into the hydrophobic cleft around Ile634

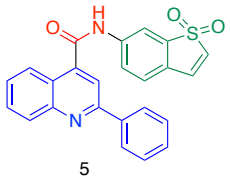
Residues of STAT3.

Predicted binding mode for compound 5

Generated using Pymol.

1. S. Becker, B. et. al, Nature 394 (1998) 145~151.
2. O. Trott. et. al, J. Comput. Chem. 31 (2010) 455~461.
3. K. Matsuno, et. al. ACS Med. Chem. Lett. 1 (2010) 371~375.
4. J. Turkson, et. al. J. Biol. Chem. 280 (2005) 32979~32988.
5. H. Song, et. al, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 4700~4705.

SAR Continued—Hydrophobic Groups



5

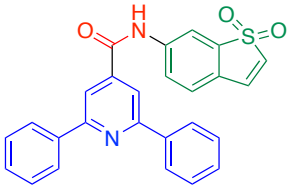
Amide linker

Heterocyclic ring

Keep for hydrogen bond

Hydrophobic groups

Change for further study



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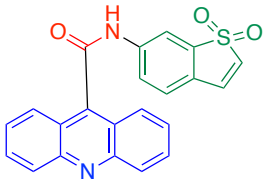

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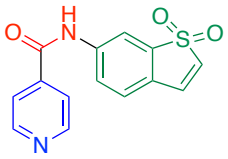

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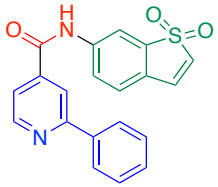

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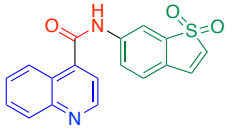
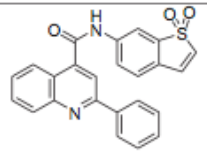
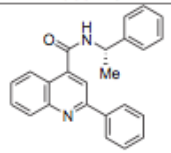
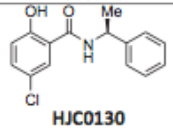
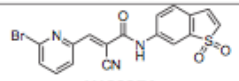
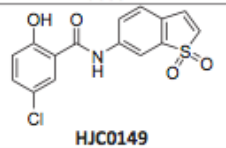
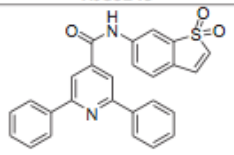
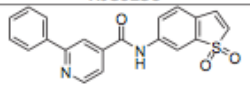


23

			5	19	20	21	22	23
IC_{50} (μM)	Breast cancer ER-Positive	MCF-7	0.1	0.65	>10	>10	3.78	2.97
	Breast cancer ER-Negative	MDA-MB-231	0.29	0.45	>10	>10	1.85	6.21
	Pancreatic cancer	AsPC1	1.25	0.12	>10	ND	1.3	6.97
		Panc-1	0.26	0.31	>10	ND	3.35	7.92

Physicochemical Analysis: cpd 5—Most Desirable

Table S1. Physicochemical parameters[1-2] of selected novel STAT3 inhibitors

Compound	Chemical Strcture	TPSA	cLogP	MW	HD (nOHNH)	HA (nON)
5	 HJC0123	76.1	4.20	412.47	1	5
8	 HJC0128	42.0	5.10	352.437	1	3
9	 HJC0130	49.3	4.04	275.735	2	3
10	 HJC0371	99.9	2.63	416.256	1	6
12	 HJC0149	83.5	2.88	335.768	2	5
19	 HJC0136	76.1	4.65	438.508	1	5
22	 HJC0430	76.1	2.95	362.41	1	5

Cellular Biological Characterization—cpd 5

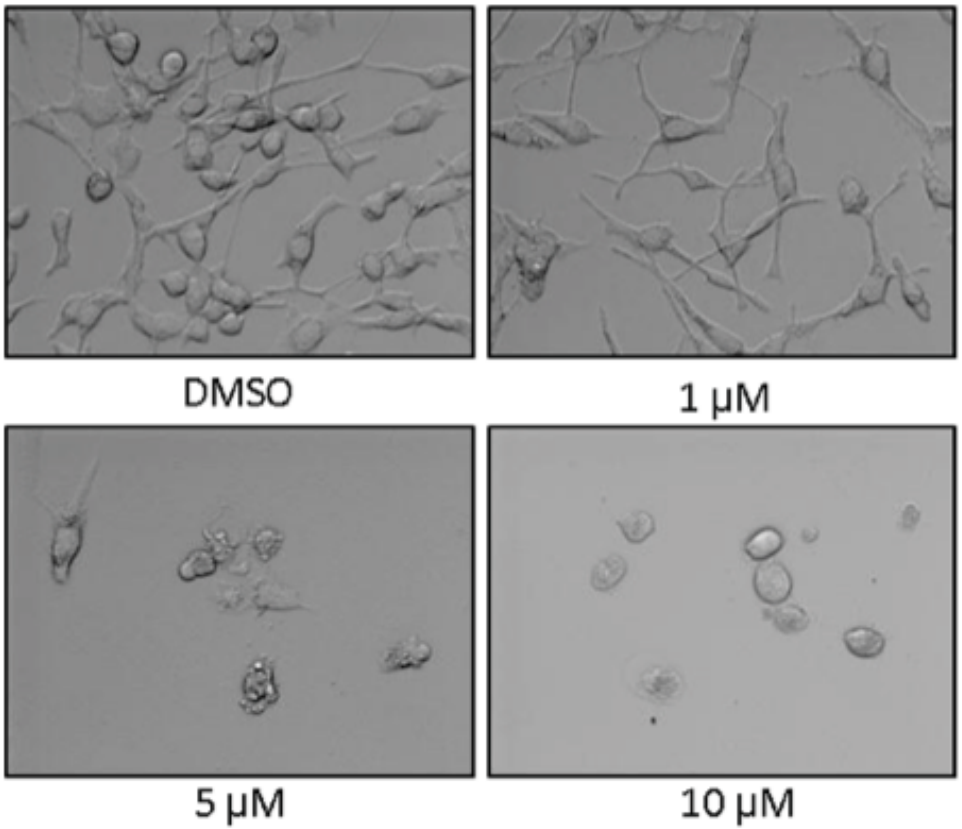
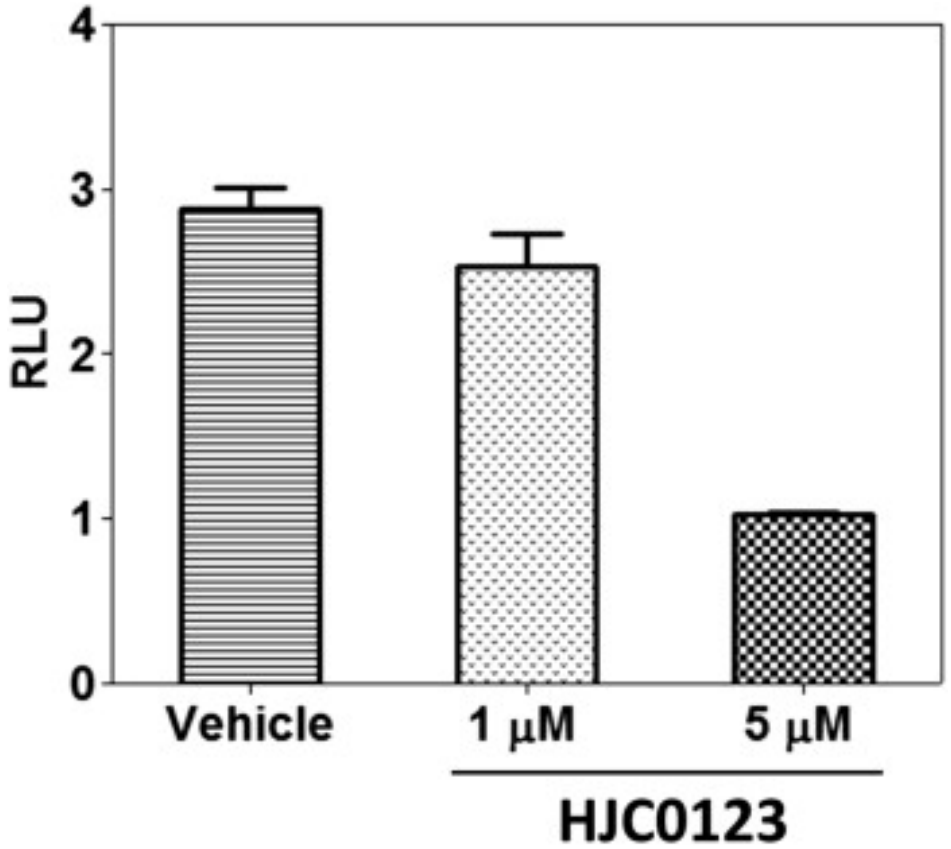


Fig. S1: Effect of **5** (**HJC0123**) on cell growth and cellular morphological change. Significantly inhibited cell proliferation and induced apoptosis.

Effect on Promoter Activity



Inhibit: 65% at 5 μM

cpd 5 acts as a potent small-molecule inhibitor of STAT3 activation

Fig 4: Compound 5 (HJC0123) inhibited the STAT3 mediated luciferase reporter activity in MDA-MB-231 cells.

Inhibitory Activity against STAT3 Pathway

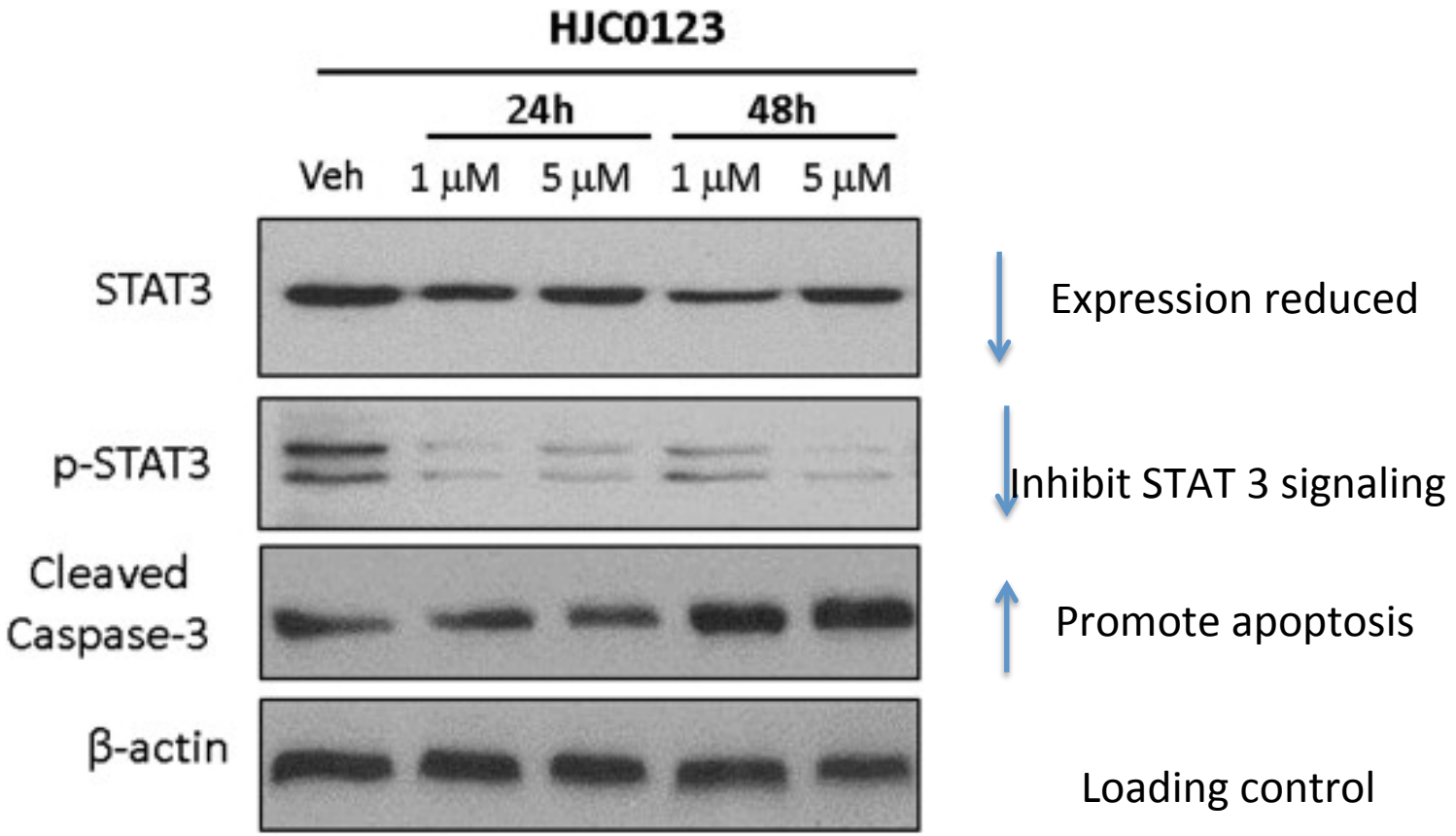
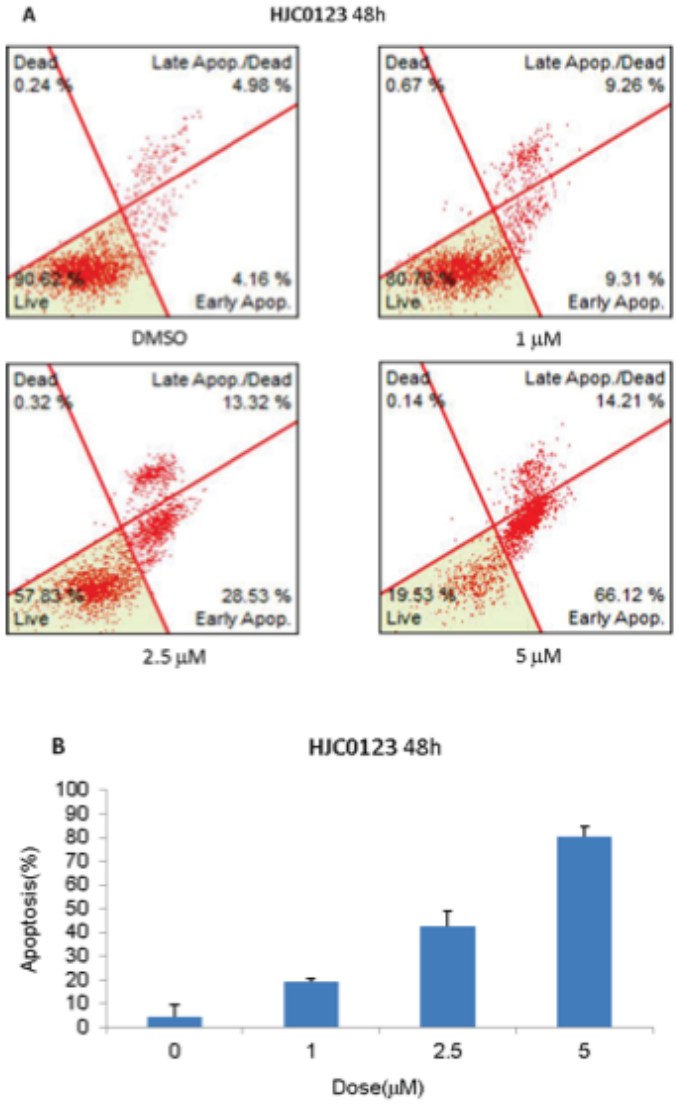


Fig. 5. Western blot analysis of biochemical markers for apoptosis induction and inhibition of STAT3 activity by compound 5 (HJC0123) in the MDA-MB-231 cell line.

Flow Cytometry—cpd 5

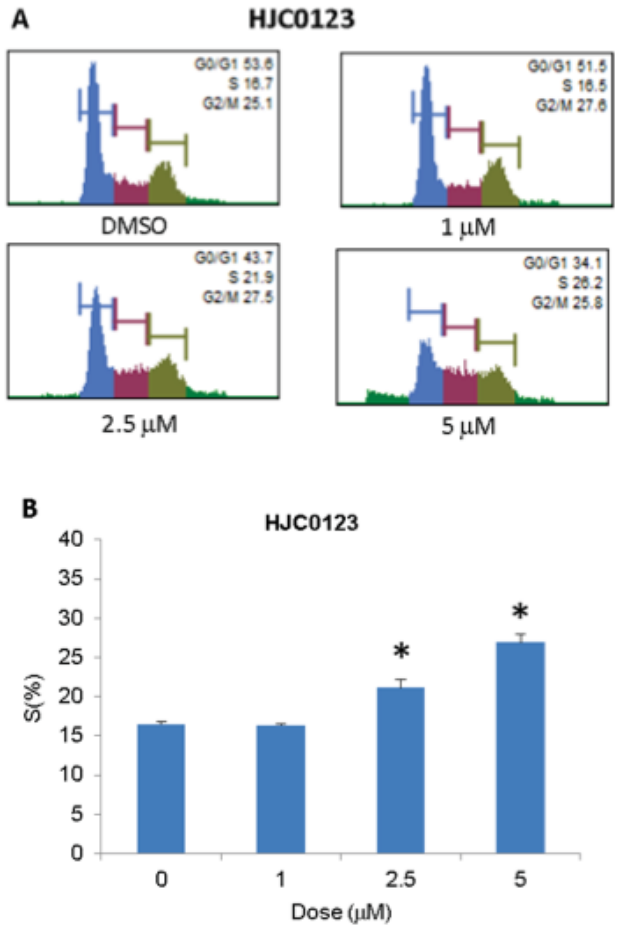


Activated apoptosis

dose-dependent manner

Figure S2.
Induction of apoptosis on MDA-MB-231 cells by **HJC0123.**

Cell Cycle Distribution Analysis — cpd 5



Arrested S phase

dose-dependent manner

Figure S3. Changes of cell cycle distribution in MDA-MB-231 cells after treatment with HJC0123.

In Vivo Biological Characterization—cpd 5

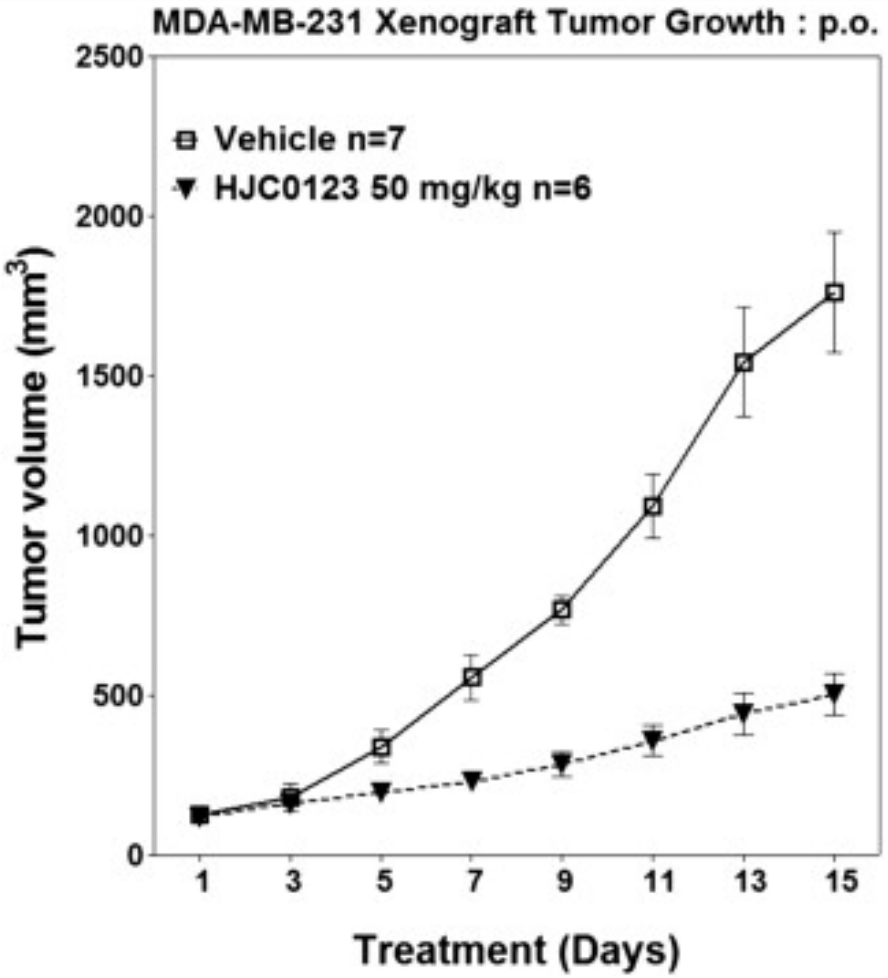
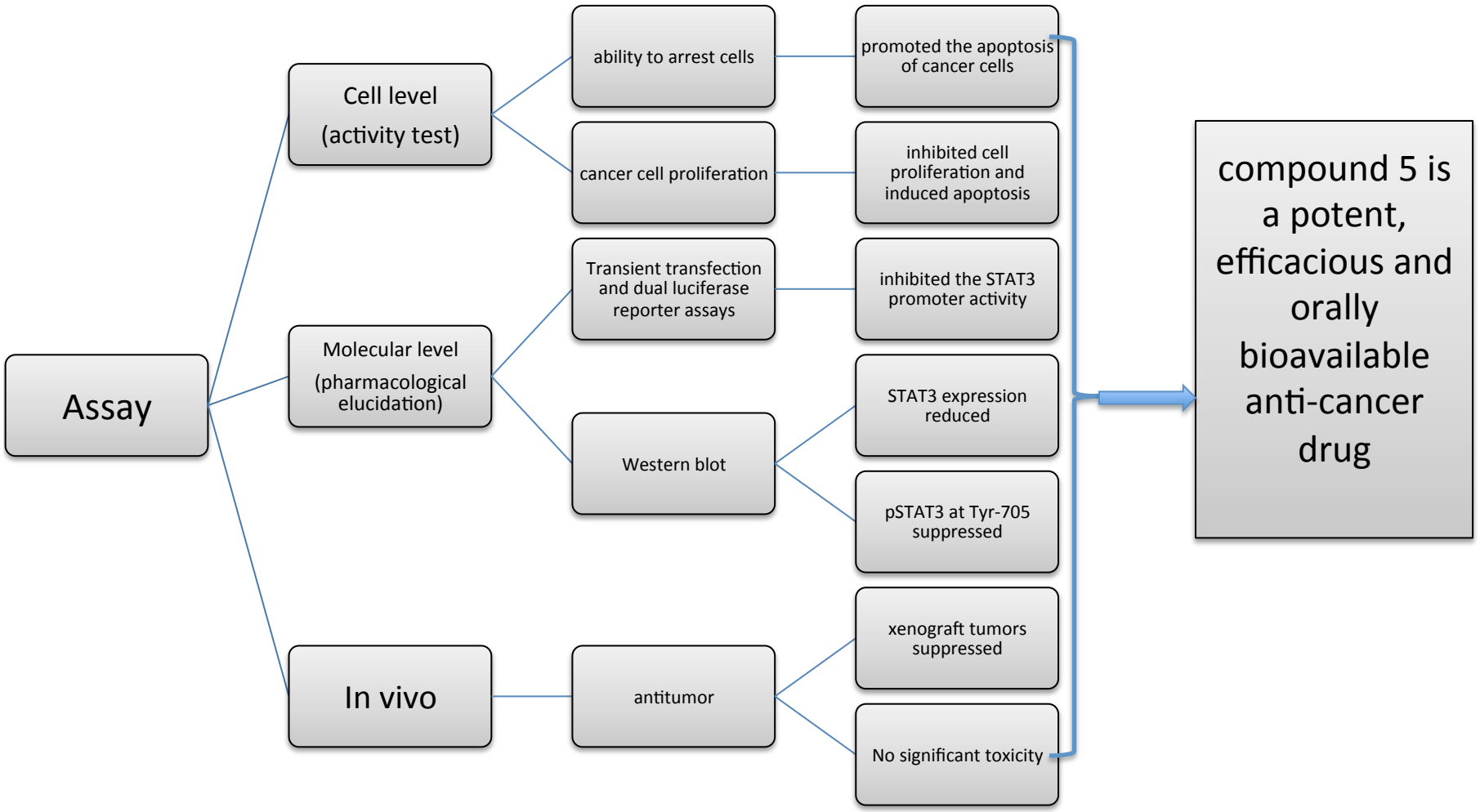


Fig. 6. In vivo efficacy of compound 5 (HJC0123) in inhibiting growth of xenograft tumors (Breast cancer MDA-MB-231) in mice (p.o.).

Assay Summary



Conclusion

FBDD

six privileged fragments

advanced chemical leads

compounds 5, 12, and 19

most potent

compound 5

In vitro

Inhibit STAT3 promoter activity

down-regulate pSTAT3

increase cleaved caspase-3

inhibit cell cycle progression and promote apoptosis

In vivo

suppress ER- negative breast cancer MDA-MB-231 xenograft tumor growth

an efficacious and orally bioavailable drug candidate for human cancer therapy.

Future work: Further modification & more extensive mechanistic study continuing