cell signaling

The Effective Use of Protein Kinase Inhibitors

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Introduction

The advent of relatively specific cell-permeable inhibitors of protein kinases in the mid-1990s has had a major impact on the study of signal transduction. The ability to rapidly suppress the cellular activity of a particular protein kinase has proved to be a powerful method for identifying the physiological substrates of these enzymes and the roles of the signaling pathways in which they participate. These compounds enter cells within minutes, so that indirect effects caused, for example, by changes in gene expression (a potential hazard when using cells deficient in a particular protein kinase), are excluded. Moreover, the use of protein kinase inhibitors avoids the need for transfection-based approaches that have the potential to give misleading results, since the fidelity of signaling can break down when components are overexpressed. Nevertheless, in order to use protein kinase inhibitors effectively it is important to realize their limitations, as well as their strengths.

An appreciation of the degree of specificity of any particular inhibitor is clearly a critical issue. There are just over 500 protein kinases encoded by the human genome, most of which belong to the same superfamily. It is therefore a challenging and difficult task to develop compounds that inhibit one particular protein kinase, without inhibiting several related enzymes. Table 2 provides current information about the specificities of 41 protein kinase inhibitors, many of which are available from Sigma-RBI, on a wide range of protein kinases.

Protein kinases in cell-based assays

Most inhibitors of protein kinases target more than one enzyme. There is, therefore, a danger that, in cell-based assays, the observed effects do not result from inhibition of the kinase of interest, but rather from inhibition of another protein kinase. In order to exclude this possibility, it is necessary to show that the effects of an inhibitor disappear in cells that express an inhibitor-resistant mutant of the kinase of interest. However, at present, the availability of such cells is very limited.[1,2]

In order to reduce this risk, it is important to examine, wherever possible, the effects of at least two structurally unrelated inhibitors of the same protein kinase. For example, kenpaullone (Product Code K 3888) and roscovitine (Product Code R 7772), which are relatively specific inhibitors of cyclin-dependent protein kinases (CDKs), also inhibit a few other protein kinases. However, the other enzymes inhibited by roscovitine are not the same as those inhibited by kenpaullone (Table 2). Thus, if identical effects are observed with roscovitine or kenpaullone, one can have greater confidence that the effects are mediated by a CDK.

For similar reasons, it is advisable to use both LiCl (Product Code L 0505) and kenpaullone to study glycogen synthase kinase-3 (GSK-3). Wortmannin (Product Code W 1628) and LY 294002 (Product Code L 9908) are used to identify potential roles of phosphoinositide 3-kinase (PI3K), PP1 or PP2 and SU6656 (Product Code S 9692) for Src family kinases. Y 27632 and HA1077 (Product Code H-139) are used for Rho kinase (ROCK) and protein kinase C-related kinase 2 (PRK2) (Tables 1 and 2).[3,4]

Even compounds that inhibit a number of protein kinases can sometimes be useful in excluding the involvement of one or more protein kinases in the control of a particular process. For example, H89 (Product Code B 1427), which inhibits isoforms of mitogen- and stress-activated kinase (MSK), but not the structurally related isoforms of ribosomal S6 kinase (RSK), has been used to provide evidence that RSKs do not mediate the growth factor-induced phosphorylation of the transcription factor cAMP-response-element-binding protein (CREB).[5] MSK isoforms were later shown to be the physiologically relevant protein kinases using cells deficient in these kinases.[6] Similarly, UCN01, which inhibits checkpoint kinase 1 (CHK1), but not CHK2, can be used to exclude the involvement of CHK2 in the control of responses to DNA damage or cell cycle checkpoints (Tables 1 and 2).[3]

It is also possible to vary the concentrations of inhibitors in the culture medium to differentially inhibit particular protein kinases. For example, at low concentrations PD184352 inhibits the classical mitogen-activated protein kinase (MAPK) cascade specifically, but at higher concentrations it also blocks the mitogen-activated protein kinase 5 (MKK5/ERK5) pathway.[7] However, the precise concentrations needed may vary from cell to cell. For this reason, it is essential to define the minimum concentration of an inhibitor required to suppress activity by 80-90% by examining the phosphorylation of a validated substrate of the protein kinase that is under investigation.

The vast majority of protein kinase inhibitors target the adenosine 5′-triphosphate (ATP)-binding site of a protein kinase. For this reason, much higher concentrations of inhibitors are generally needed to suppress the activity of a protein kinase in cells (where the ATP concentration is in the millimolar range), compared to the amounts required for inhibition *in vitro* (where assays are performed at much lower ATP concentrations, typically 0.01-0.1 mM). There are, however, a few inhibitors that are actually more potent in cell-based assays than they are *in vitro.* For example, PD 98059 (Product Code P-215) and U0126 (Product Code U-120), which are non-competitive inhibitors of mitogen-activated protein kinase kinase 1 (MKK1), bind much more strongly to the dephosphorylated, inactive form of this protein kinase than the phosphorylated, active enzyme.

These compounds prevent the conformational change required for activation of MKK1 and therefore suppress the classical MAPK cascade at much lower concentrations than those needed to inhibit activated MKK1 *in vitro*.[3,8] Similarly, lithium ions, a relatively specific inhibitor of GSK-3, compete for binding with magnesium ions. The free concentration of magnesium ions in cells is less than 0.5 mM, much lower than the concentration used to assay GSK-3 routinely (10 mM, see Table 1). For this reason, lithium ions inhibit GSK-3 more potently in cells than *in vitro*.[3]

In recent years, potent and highly specific inhibitors of a variety of protein kinases have been developed by several pharmaceutical companies. Many have entered human clinical trials and, in two cases (Glivec and Iressa), have been approved for the treatment of different types of cancer.[9] Over the next ten years one can therefore expect that many more protein kinase inhibitors will become available to the scientific community, which should advance at an even faster pace our understanding of the function of these enzymes.

References

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Table 1. How to use the more specific inhibitors of protein kinases in cell-based assays

Inhibitor	Specificity	Target Kinase(s)***	Concentration to use in Culture Medium (µM)*
Rapamycin **(R 0395)**	Very high	mTOR	0.1
PD 98059 **(P-215)**	High	MKK1	50
PD 184352**	High	MKK1	1-2
PD 184352**	High	MKK1, MKK5	10-20
U0126 **(U-120)**	High	MKK1, MKK5	5-10
SB-203580 **(S 8307)**	High	SAPK2a/p38α, SAPK2b/p38β2	1-10
SB-202190 **(S 7067)**	High	SAPK2a/p38α, SAPK2b/p38β2	1-5
KN62 **(I 2142)**	High	CaM-KII, other CaM-Ks	10
Wortmannin **(W 1628)**	High	PI3K	0.1
LY 294002 **(L 9908)**	Quite high	PI3K	50-100
Y27632	Quite high	ROCK, PRK2	10-20
HA1077 **(H-139)**	Medium	ROCK, PRK2	10-100
LiCl **(L 0505)**	Quite high	GSK-3	10
Kenpaullone **(K 3888)**	Quite high	GSK-3, CDKs	10
Roscovitine **(R 7772)**	High	CDKs	10-100
PP1	Quite high	Src, Fyn, Lck	0.1-1.0
PP2	Quite high	Src, Fyn, Lck	0.1-1.0
SU6656 **(S 9692)**	Medium	Src, Fyn, Lck	10-50
ML7 **(I 2764)**	Quite high	Sm-MLCK	50-100
H89** **(B 1427)**	Medium	PKA	5-10
H89** **(B 1427)**	Medium	PKA, MSKs, but not RSKs	10-25
Ro 31-8220** **(R-136)**	Medium	Conventional PKCs	1
Ro 31-8220** **(R-136)**	Medium	PKCs, MSKs, RSKs, etc	5
UCN01	Quite low	PDK1 and CHK1, but not CHK2	0.3-1

* The suggested concentrations are only guidelines. The optimal concentrations can vary and need to be defined for each cell used, as discussed in the text. Sigma-RBI product numbers are shown in red.

**Depending on the concentration range at which they are used, these kinase inhibitors can be used to target different groups of protein kinases.

***Kinase names are provided in the Table 1 legend.

Table 2. Inhibition of protein kinases by various inhibitors. *Results indicate percent activity observed in the presence of inhibitor expressed as a percentage of control incubations in the absence of inhibitor. Data are the means of duplicate determinations. Data highlighted in boxes indicate instances when a given inhibitor reduced kinase activity to ≤ 25% of control values. Assays were carried out at a magnesium ion concentration of 10 mM and an ATP concentration of 0.1 mM. Column headers indicate the protein kinase inhibitors tested, together with the concentrations at which they were used and their Sigma-RBI product numbers in red.*

Protein kinase	H89 (B 1427) (10 µM)	Y 27632 (10 µM)	HA1077 (H-139) (20 µM)	Rottlerin (R 5648) (20 µM)	KN62 (I 2142) (10 µM)	U0126 (U-120) (10 µM)	PD 184352 (10 µM)	PD 98059 (P-215) (50 µM)	SB-203580 (S 8307) (10 µM)	SB-202190 (S 7067) (10 µM)	Wortmannin (W 1628) (1 µM)	LY 294002 (L 9908) (50 µM)	Quercetin (Q 0125) (20 µM)	Rapamycin (R 0395) (1 µM)	LiCl (L 0505) (10 mM)	KCl (P 5405) (10 mM)	Ro 31-8220 (R-136) (1 µM)	Bis-1 (B 6292) (1 µM)	Bis-3 (B 3181) (1 µM)
(A) Core panel																			
MKK1	90	103	89	106	101	56	**5**	89	99	93	96	101	94	99	116	109	94	94	90
MAPK2/ERK2	87	94	94	139	92	92	107	85	85	89	90	114	113	90	107	102	97	98	107
JNK1α1/SAPK1c	97	98	96	49	104	96	102	111	101	93	97	108	101	98	92	89	95	91	100
JNK/SAPK1c																			
SAPK2a/p38	99	94	93	111	95	75	100	85	**2**	**0**	86	98	138	93	108	102	84	88	104
SAPK2b/p38β2	97	107	97	127	98	90	119	95	**10**	**3**	74	98	150	88	96	104	97	92	115
SAPK3/p38γ	106	100	87	146	95	100	100	96	96	80	75	97	132	100	99	108	92	89	116
SAPK4/p38δ	105	95	103	130	110	111	98	94	93	87	79	94	103	82	84	99	104	113	133
MAPKAP-K1a																			
MAPKAP-K1b	**16**	72	37	79	89	88	86	93	83	95	92	70	**20**	95	95	78	**2**	**9**	**2**
MAPKAP-K2	99	99	90	**5**	59	102	98	95	93	97	102	74	90	125	72	98	103	90	97
MSK1	**3**	57	**19**	38	81	104	118	86	86	88	99	83	37	104	104	105	**2**	**21**	**9**
PRAK	81	104	91	**6**	36	93	71	108	112	88	85	68	51	74	76	104	96	98	89
PKA	**2**	91	35	**17**	94	95	105	106	96	66	97	91	104	104	96	96	70	99	87
PKCα	79	98	86	95	95	92	99	93	89	92	100	91	70	99	98	97	**3**	**4**	**7**
PDK1	104	115	92	36	70	99	85	86	89	87	88	76	81	110	105	98	84	85	69
PKBα	**17**	90	88	27	67	79	89	82	62	53	96	60	99	91	95	96	73	77	99
SGK	**25**	109	92	81	78	91	111	90	83	98	101	72	35	108	99	100	**21**	63	26
S6K1	**0**	94	32	98	93	92	86	100	87	75	106	81	**25**	109	95	101	**6**	32	**18**
GSK-3β	107	92	90	**13**	38	105	83	101	66	61	85	53	30	89	58	99	**5**	50	46
ROCK-II	**0**	**13**	**7**	88	88	94	107	80	77	61	91	104	55	92	101	102	92	90	89
AMPK	**19**	95	77	98	97	85	89	97	96	94	106	103	**16**	106	106	105	42	54	**23**
CK1																			
CK2	104	98	102	103	103	107	96	87	97	93	98	**18**	**19**	104	73	112	104	101	106
PHK	51	81	58	63	106	101	117	87	104	91	100	44	32	103	96	93	57	54	**20**
LCK	76	109	94	70	92	87	99	85	32	37	95	85	83	102	99	105	79	86	86
CHK1	**21**	99	82	107	104	95	104	99	95	95	99	90	56	102	96	97	42	60	40
CHK2																			
CSK																			
CDK2/Cyclin A																			
DYRK1A																			
PKG																			
(B) Other kinases																			
CAM-KII					**0**														
SkMLCK			93								104								
SmMLCK			93		96						**4**								
PKCδ				101															
MKK3	95					114									94	109			
MKK4	80					81									87	94			
MKK6	86					79									108	113			
MKK7	91					89									100	102			
PI3K											**0**	**13**	**18**						
PRK2		**6**	**15**																

Abbreviations

AMPK: AMP-acitvated protein kinase
CaM-KII: Calcium/Calmodulin protein kinase II
CDK2/Cyclin A: Cyclin-dependent kinase 2/Cyclin A complex
CHK1: Checkpoint kinase 1
CHK2: Checkpoint kinase 2
CK1: Casein kinase 1
CK2: Casein kinase 2
CSK: COOH-terminal Src kinase
DYRK1A: Dual-specificity tyrosine phosphorylation-regulated kinase 1A
GSK-3β: Glycogen synthase kinase-3β
JNK/SAPK1c:: c-jun N-terminal kinase
JNK1α1/SAPK1c: c-jun N-terminal kinase
LCK: T-cell specific kinase; lymphocyte-specific kinase
MAPK2/ERK2: Mitogen-activated protein kinase 2
MAPKAP-K1a: Mitogen-activated protein kinase-activated protein kinase-1a
MAPKAP-K1b: Mitogen-activated protein kinase-activated protein kinase-1b
MAPKAP-K2: Mitogen-activated protein kinase-activated protein kinase-2
MKK1: Mitogen-activated protein kinase kinase 1
MKK3: Mitogen-activated protein kinase kinase 3

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Bis-4 (B 3306) (1 µM)	Bis-5 (B 3431) (1 µM)	Bis-8 (B 3806) (1 µM)	Bis-10 (B 3931) (1 µM)	Gö 6976 (1 µM)	K252c (10 µM)	KT 5720 (K 3761) (10 µM)	UCN01 (1 µM)	Roscovitine (R 7772) (10 µM)	Purvalanol A (P 4484) (10 µM)	Indirubin-3'-monoxime (I 0404) (10 µM)	Kenpaullone (K 3888) (10 µM)	Alsterpaullone (A 4847) (10 µM)	PP1 (10 µM)	PP2 (10 µM)	PP3 (10 µM)	SU 6656 (S 9692) (10 µM)	SP 600125 (S 5567) (10 µM)	KT 5823 (K 1388) (10 µM)	EGCG (E 4143) (10 µM)	ML-7 (I 2764) (20 µM)	ML-9 (C 1172) (100 µM)
84	99	99	9	64	59	20	90	93	80	94	103	96	52	55	89	82	89	99	94	102	95
86	101	105	106	34	37	57	87	81	26	104	70	64	75	61	92	102	55	106	53	85	101
95	100	95	103	88	82	81	98														
								91	84	21	128	89	98	98	99	103	38	99	76	97	102
90	101	115	117	92	100	103	92	96	101	83	84	79	13	21	100	70	86	102	105	100	94
88	104	104	102	81	88	98	91	104	117	45	108	64	22	26	84	90	75	100	87	89	98
83	99	107	116	72	64	95	76	94	105	74	87	55	92	90	83	75	82	103	85	95	98
90	88	129	112	96	93	95	91	106	98	65	104	73	102	96	95	78	98	105	60	75	86
								69	15	20	86	82	69	114	115	27	46	105	109	99	44
54	92	4	5	5	29	23	5														
113	124	104	105	80	83	62	90	85	97	69	110	40	96	106	98	96	72	71	79	93	106
56	103	13	5	2	62	39	19	92	88	40	92	98	55	57	93	94	51	74	88	59	14
96	85	102	101	76	50	58	77	87	76	38	83	78	85	85	97	95	39	83	10	78	91
93	95	94	90	85	99	39	39	93	82	80	109	79	76	74	89	73	82	102	109	85	71
57	104	14	1	7	60	87	1	101	97	68	120	93	80	66	100	90	79	102	76	85	80
95	109	107	122	18	6	7	0	104	103	27	52	41	97	99	82	94	62	114	50	107	113
101	91	98	104	87	65	36	31	89	88	62	86	61	97	77	108	100	95	83	87	88	76
101	96	28	34	77	28	29	63	104	91	9	92	42	94	111	94	70	22	72	104	101	84
87	110	29	12	12	49	66	28	94	79	20	117	64	43	70	93	71	22	90	87	74	27
49	109	21	42	33	84	39	83	106	84	6	6	4	86	113	99	83	60	52	78	107	107
100	107	106	103	41	19	84	22	94	57	15	53	45	65	75	93	43	59	97	53	74	23
96	102	60	71	50	30	22	1	95	59	6	72	36	93	84	94	14	26	90	101	82	61
								48	75	38	99	86	8	6	54	66	10	83	105	106	103
99	98	103	104	101	67	95	77	101	95	82	99	67	96	90	76	93	63	98	105	83	69
90	100	58	60	1	0	0	0	80	36	102	110	77	70	93	99	21	34	99	78	85	79
91	96	86	98	58	68	46	7	97	22	11	15	19	0	1	77	8	53	94	89	87	116
91	104	71	50	3	4	13	3	95	78	38	65	34	91	93	96	66	39	93	79	94	103
							69														
								109	20	105	86	90	3	3	96	89	71	93	76	94	96
								7	2	18	22	5	75	68	84	54	20	94	97	73	75
								15	6	62	116	102	101	94	60	23	16	112	9	66	38
																		109			
																				63	54
																				10	25

MKK4: Mitogen-activated protein kinase kinase 4
MKK6: Mitogen-activated protein kinase kinase 6
MKK7: Mitogen-activated protein kinase kinase 7
MSK1: Mitogen- and stress-activated protein kinase-1
mTOR: Mammalian target of rapamycin
PDK1: 3-Phosphoinositide-dependent protein kinase 1
PHK: Phosphorylase kinase
PI3K: Phosphoinositide 3-kinase
PKA: Protein kinase A
PKBα: Protein kinase Bα
PKCα: Protein kinase Cα
PKCδ: Protein kinase Cδ
PKG: Protein kinase G
PRAK: p38-regulated/activated kinase
PRK2: PKC-related kinase 2
ROCK: Rho-associated protein kinase
ROCK-II: Rho-associated coiled-coil forming protein kinase-II
RSK: Ribosomal S6 kinase
S6K1: p70 S6 kinase
SAPK2a/p38: p38 kinase
SAPK2b/p38β2: p38 kinase
SAPK3/p38γ: p38 kinase
SAPK4/p38δ: p38 kinase
SGK: Serum and glucocorticoid–induced kinase
Sk-MLCK: Skeletal myosin light chain kinase
Sm-MLCK: Smooth myosin light chain kinase