The specificities of protein kinase inhibitors: an update

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We have previously examined the specificities of 28 commercially available compounds, reported to be relatively selective inhibitors of particular serine/threonine-specific protein kinases [Davies, Reddy, Caivano and Cohen (2000) *Biochem. J.* **351**, 95–105]. In the present study, we have extended this analysis to a further 14 compounds. Of these, indirubin-3'-monoxime, SP 600125, KT 5823 and ML-9 were found to inhibit a number of protein kinases and conclusions drawn from their use in cell-based assays are likely to be erroneous. Kenpaullone, Alsterpaullone, Purvalanol, Roscovitine, pyrazolopyrimidine 1 (PP1), PP2 and ML-7 were more specific, but still inhibited two or more protein kinases with similar potency. Our results suggest that the combined use of Roscovitine and Kenpaullone may be useful for identifying

substrates and physiological roles of cyclin-dependent protein kinases, whereas the combined use of Kenpaullone and LiCl may be useful for identifying substrates and physiological roles of glycogen synthase kinase 3. The combined use of SU 6656 and either PP1 or PP2 may be useful for identifying substrates of Src family members. Epigallocatechin 3-gallate, one of the main polyphenolic constituents of tea, inhibited two of the 28 protein kinases in the panel, dual-specificity, tyrosine-phosphorylated and regulated kinase 1A (DYRK1A; $IC_{50} = 0.33 \mu M$) and p38-regulated/activated kinase (PRAK; $IC_{50} = 1.0 \mu M$).

Key words: Kenpaullone, protein kinase inhibitors, protein phosphorylation, Roscovitine, SP 600125, Src.

INTRODUCTION

In recent years, there has been considerable interest in the development of small cell-permeable inhibitors of protein kinases. Many such compounds are now undergoing human clinical trials for the treatment of cancer, chronic inflammatory diseases and other indications, and a few have already been approved for clinical use (reviewed in [1]).

There is also considerable interest in the exploitation of specific protein-kinase inhibitors for the study of cell signalling. Indeed, several such compounds have already had a major impact in the field, especially PD 98059 [2] and U0126 [3], which suppress activation of the classical mitogen-activated protein kinase (MAPK) cascade by inhibiting the activation of MAPK kinase-1 [4], SB 203580 [5] and SB 202190 [6], which inhibit stress-activated protein kinase (SAPK) 2a (also called p38), and rapamycin, which inhibits the protein kinase mTOR (mammalian target of rapamycin) [7]. In the case of rapamycin [7] and SB 203580 [8], the *in vivo* specificity of these compounds has been validated by the demonstration that many of their effects disappear in cells that express drug-resistant mutants of the protein kinases that they inhibit.

Many other compounds, reported to be specific inhibitors of protein kinases, are available commercially. However, when we examined the specificities of 28 of these compounds against a large panel of protein kinases, many were found to be so non-specific as to render meaningless any conclusions drawn from their use [6]. Since the publication of this study, we have been overwhelmed with requests to extend our studies to additional compounds and to make the results available to the scientific

community. In the present paper, we therefore update our earlier report by profiling the specificities of a further 14 compounds, which are being widely used to study cell signalling.

MATERIALS AND METHODS

Histone H1 was purchased from Sigma (Poole, Dorset, U.K.) and peptides were synthesized by Dr Graham Bloomberg (Department of Biochemistry, University of Bristol, Bristol, U.K.). Alsterpaullone, Kenpaullone, indirubin-3'-monoxime, SU 6656, KT 5823, ML-7, ML-9, pyrazolopyrimidine 2 (PP2) and PP3 were purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA, U.S.A.). PP1 was obtained from Alexis Corp. (U.K.) Ltd (Nottingham, U.K.). SP 600125 was from Biomol (Plymouth Meeting, PA, U.S.A.). Epigallocatechin 3-gallate (EGCG) was from Sigma. The sources of other reagents have been detailed previously in [6].

Production of protein kinases

Human dual-specificity, tyrosine-regulated protein kinase (DYRK) 1A, human C-terminal Src kinase (CSK) and rat casein kinase (CK) 1 δ were expressed as active glutathione S-transferase (GST) fusion proteins in *Escherichia coli* and purified by affinity chromatography on glutathione-Sepharose by the Protein Production team in the Division of Signal Transduction Therapy at Dundee. The cDNA clones encoding DYRK1A, CK1 δ and CSK were gifts from Dr Walter Becker (Institute for Pharmacology and Toxicology, Aachen, Germany), Dr David Meek (Biomedical Research Centre, University of Dundee, Dundee, Scotland, U.K.) and AstraZeneca respectively. Human cyclin-dependent protein

Abbreviations used: AMPK, AMP-activated protein kinase; CDK, cyclin-dependent protein kinase; CK, casein kinase; CSK, C-terminal Src kinase; DYRK, dual-specificity, tyrosine-phosphorylated and regulated kinase; EGCG, epigallocatechin 3-gallate; ERK, extracellular-signal-regulated kinase; GSK3, glycogen synthase kinase 3; GST, glutathione S-transferase; JNK, c-Jun N-terminal kinase; LCK, lymphocyte kinase; MAPK, mitogen-activated protein kinase; MLCK, myosin light chain kinase; MSK1, mitogen- and stress-activated protein kinase 1; PHK, phosphorylase kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PP, pyrazolopyrimidine; PRAK, p38-regulated/activated kinase; ROCK-II, Rho-dependent protein kinase II; RSK1, ribosomal S6 kinase 1; SAPK, stress-activated protein kinase; SGK, serum- and glucocorticoid-induced kinase; S6K1, p70 ribosomal protein S6 kinase.

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Table 1 Inhibition of protein kinases by commercially available inhibitors

The inhibitor concentrations used are shown in parentheses. Results are presented as kinase activity as a percentage of that in control incubations (means of duplicate determinations). ATP was present at 0.1 mM in all assays. All kinases used were of human origin apart from MKK1 (rabbit), MAPK2 (mouse), MAPKAP-K1a (rat), PKA (cow), ROCK-II (rat), AMPK (rat), PHK (rabbit), CK1 (rat) and DYRK1A (rat). CHK1, checkpoint kinase 1; MAPKAP-K, MAPK-activated protein kinase; MKK1, MAPK kinase 1; PDK1, 3-phosphoinositide-dependent protein kinase 1; PHK, phosphorylase kinase; PKB α , protein kinase B α .

Protein Kinase	Roscovitine (10 μ M)	Purvalanol A (10 μ M)	Indirubin-3'-monoxime (10 μ M)	Kenpaullone (10 μ M)	Alsterpaullone (10 μ M)
MKK1	93 $\pm$ 8	80 $\pm$ 6	94 $\pm$ 4	103 $\pm$ 8	96 $\pm$ 4
MAPK2/ERK2	81 $\pm$ 7	26 $\pm$ 1	104 $\pm$ 1	70 $\pm$ 3	64 $\pm$ 3
JNK/SAPK1c	91 $\pm$ 5	84 $\pm$ 5	21 $\pm$ 5	128 $\pm$ 3	89 $\pm$ 1
SAPK2a/p38	96 $\pm$ 7	101 $\pm$ 1	83 $\pm$ 0	84 $\pm$ 0	79 $\pm$ 4
SAPK2b/p38 β	104 $\pm$ 4	117 $\pm$ 6	45 $\pm$ 4	108 $\pm$ 2	64 $\pm$ 5
SAPK3/p38 γ	94 $\pm$ 3	105 $\pm$ 7	74 $\pm$ 4	87 $\pm$ 4	55 $\pm$ 4
SAPK4/p38 δ	106 $\pm$ 8	98 $\pm$ 8	65 $\pm$ 2	104 $\pm$ 2	73 $\pm$ 3
MAPKAP-K1a	69 $\pm$ 3	15 $\pm$ 7	20 $\pm$ 1	86 $\pm$ 3	82 $\pm$ 3
MAPKAP-K2	85 $\pm$ 3	97 $\pm$ 0	69 $\pm$ 4	110 $\pm$ 1	40 $\pm$ 4
MSK1	92 $\pm$ 5	88 $\pm$ 1	40 $\pm$ 2	92 $\pm$ 6	98 $\pm$ 7
PRAK	87 $\pm$ 6	76 $\pm$ 2	38 $\pm$ 6	83 $\pm$ 4	78 $\pm$ 8
PKA	93 $\pm$ 2	82 $\pm$ 3	80 $\pm$ 1	109 $\pm$ 8	79 $\pm$ 1
PKC α	101 $\pm$ 2	97 $\pm$ 4	68 $\pm$ 7	120 $\pm$ 4	93 $\pm$ 2
PDK1	104 $\pm$ 5	103 $\pm$ 5	27 $\pm$ 1	52 $\pm$ 7	41 $\pm$ 0
PKB α	89 $\pm$ 3	88 $\pm$ 7	62 $\pm$ 2	86 $\pm$ 8	61 $\pm$ 5
SGK	104 $\pm$ 5	91 $\pm$ 3	9 $\pm$ 0	92 $\pm$ 7	42 $\pm$ 2
S6K1	94 $\pm$ 6	79 $\pm$ 2	20 $\pm$ 2	117 $\pm$ 8	64 $\pm$ 2
GSK3 β	106 $\pm$ 4	84 $\pm$ 6	6 $\pm$ 2	6 $\pm$ 4	4 $\pm$ 1
ROCK-II	94 $\pm$ 4	57 $\pm$ 8	15 $\pm$ 3	53 $\pm$ 7	45 $\pm$ 3
AMPK	95 $\pm$ 2	59 $\pm$ 8	6 $\pm$ 2	72 $\pm$ 1	36 $\pm$ 2
CHK1	95 $\pm$ 1	78 $\pm$ 0	38 $\pm$ 5	65 $\pm$ 3	34 $\pm$ 1
CK2	101 $\pm$ 6	95 $\pm$ 0	82 $\pm$ 1	99 $\pm$ 5	67 $\pm$ 0
PHK	80 $\pm$ 2	36 $\pm$ 2	102 $\pm$ 1	110 $\pm$ 4	77 $\pm$ 7
LCK	97 $\pm$ 6	22 $\pm$ 5	11 $\pm$ 3	15 $\pm$ 0	19 $\pm$ 0
CSK	109 $\pm$ 4	20 $\pm$ 2	105 $\pm$ 3	86 $\pm$ 8	90 $\pm$ 1
CDK2/Cyclin A	7 $\pm$ 4	2 $\pm$ 0	18 $\pm$ 5	22 $\pm$ 2	5 $\pm$ 2
CK1	48 $\pm$ 4	75 $\pm$ 1	38 $\pm$ 0	99 $\pm$ 3	86 $\pm$ 2
DYRK1A	15 $\pm$ 0	6 $\pm$ 0	62 $\pm$ 3	116 $\pm$ 6	102 $\pm$ 1

kinase-2 (CDK2) complexed to cyclin A3 was provided by Dr Jane Endicott (University of Oxford, Oxford, U.K.). The production of active forms of the other protein kinases used in this study has been detailed in [6]. Unless stated otherwise, they were of human origin and expressed as either GST fusion proteins in *E. coli* (purified by Miss Fiona Douglas in this Division), or as hexahistidine (His₆)-tagged proteins in insect Sf21 cells (purified by Dr Andrew Paterson, Miss Carla Clark and Miss Kate Winstanley in this Division). cGMP-dependent protein kinase (PKG) was obtained from Dr Suzanne Lohmann (University of Würzburg, Würzburg, Germany).

Assay of protein kinases

All protein kinase assays (25 μ l) were carried out at room temperature (21 °C) and were linear with respect to time and enzyme concentrations under the conditions used. Assays were performed for 40 min using a Biomek 2000 Laboratory Automation Workstation in a 96-well format (Beckman Instruments, Palo Alto, CA, U.S.A.). The concentrations of magnesium acetate and [γ -³²P]ATP (800 cpm/pmol) in the assays were 10 mM and 0.1 mM, respectively, unless stated otherwise. Assays were initiated with MgATP and stopped by the addition of 5 μ l of 0.5 M orthophosphoric acid. Aliquots were then spotted on to P30 filtermats, washed four times in 75 mM phosphoric acid to remove ATP, once in methanol, then dried and counted for radioactivity.

CSK (20 mU) diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01 % (w/v) Brij35, 5 % (v/v) glycerol, 0.1 % (v/v) 2-

mercaptoethanol, 1 mg/ml bovine serum albumin was assayed using the peptide KVEKIGEGTYGVVYK in an incubation containing 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.25 mM peptide substrate, 10 mM magnesium acetate and 0.1 mM [γ -³²P]ATP (500–1000 cpm/pmol).

CDK2/cyclin A (5–20 m-units), diluted in 50 mM Hepes, pH 7.5, 1 mM dithiothreitol, 0.02 % (w/v) Brij 35 and 100 mM NaCl was assayed in the same buffer using Histone H1 (1 mg/ml) as a substrate.

DYRK1A (5–20 m-units) was diluted in 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA and 0.1 % (v/v) 2-mercaptoethanol and assayed against Woodtide (the peptide KKISGRSLPIMTEQ) in an incubation containing 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 0.1 % (v/v) 2-mercaptoethanol and 350 μ M substrate peptide.

CK1 δ (5–20 m-units), diluted in 20 mM Hepes, pH 7.5, 0.15 M NaCl, 0.1 mM EGTA, 0.1 % (v/v) Triton X-100, 5 mM dithiothreitol, 50 % (v/v) glycerol was assayed against the peptide RRRDLHDEEDEAMSITA in an incubation containing 20 mM Hepes, pH 7.5, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1 % (v/v) Triton X-100 and 0.5 mM substrate peptide.

PKG (5–20 m-units), diluted in 50 mM Tris/HCl, pH 7.5, 0.1 mM EGTA, 0.1 % (v/v) 2-mercaptoethanol and 0.1 mg/ml BSA was assayed in the same buffer using the peptide KEAKE-KRQEIAKRRRLSSLRASSTKSGGSQK as a substrate, which is similar to the sequence near the C-terminus of ribosomal protein S6.

Other protein kinases were assayed as described previously in [6].

RESULTS

Inhibitors of CDKs and glycogen synthase kinase 3 (GSK3)

Roscovitine and Purvalanol A

Olomoucine was one of the first CDK inhibitors to be developed [9]. Two of its derivatives, Roscovitine [10] and Purvalanol [11], are more potent, ATP-competitive inhibitors of CDK1, CDK2 and CDK5 and have been used extensively to inhibit these protein kinases in cell-based assays.

We found that Roscovitine and Purvalanol inhibited CDK2 activity (Table 1) with IC_{50} values of 0.25 μ M and 0.1 μ M respectively (Table 1 and Table 2), in our standard assay, which is conducted at 0.1 mM ATP. Purvalanol, like most protein-kinase inhibitors, competes with ATP and the greater potency of Purvalanol reported by others can be explained by the use of lower ATP concentrations in their assays [11]. Roscovitine also inhibited DYRK1A (Table 1) with an IC_{50} of 3.1 μ M and CK1 δ with an IC_{50} of 17 μ M (Table 2), but had little effect on the other protein kinases in the panel. Purvalanol A was a 2.5-fold more potent inhibitor of CDK2, but also inhibited DYRK1A potently (Table 1 and Table 2) and a number of other protein kinases in the low micromolar range. These included ribosomal S6 kinase 1 (RSK1), extracellular-signal-regulated kinase (ERK) 2 and the protein tyrosine kinases lymphocyte kinase (LCK) and CSK. The inhibition of ERK1 and ERK2 was also reported by others [12] after the present paper had been submitted.

Indirubin-3'-monoxime

Indirubin dyes are thought to be the active ingredient of Danggui Longhui Wan, a traditional Chinese medicine which has been used for hundreds of years to treat chronic diseases, such as cancer. These compounds were originally reported to be potent ATP-competitive inhibitors of CDK1, CDK2 and CDK5 and to cause cell-cycle arrest [13], but were subsequently reported to inhibit GSK3 β with similar or greater potency [14].

In our assays, indirubin-3'-monoxime inhibited GSK3 β and CDK2 with IC_{50} values of 0.19 μ M and 0.59 μ M respectively. The greater potency of indirubin-3'-monoxime reported by another laboratory is explained by the lower ATP concentrations in their assays [14]. However, indirubin-3'-monoxime inhibited many other protein kinases with similar or only slightly lower potency, such as serum- and glucocorticoid-induced kinase (SGK), AMP-activated protein kinase (AMPK) and LCK (Table 1 and Table 2).

Kenpaullone and Alsterpaullone

Like the Indirubin dyes, the Paullones were first reported to be ATP-competitive inhibitors of CDK1/cyclin B [15] and subsequently to inhibit GSK3 and other CDKs, such as CDK2 and CDK5 [16]. The inhibition of CDK1/cyclin B may underlie the anti-tumour effects of Alsterpaullone [15], whereas suppression of the phosphorylation of tau by Alsterpaullone [16] and Kenpaullone [14] may result from the inhibition of GSK3 β and/or CDK5. The inhibition of CDK5 is also thought to underlie the suppression of DARPP-32 (dopamine and adenosine 3',5'-cyclic monophosphate-regulated phosphoprotein of 32 kDa) phosphorylation by Alsterpaullone and Kenpaullone in mouse striatal slices [14,16].

In our assays, Kenpaullone inhibited GSK3 β and CDK2 with IC_{50} values of 0.23 μ M and 0.67 μ M respectively, but LCK, a member of the Src family of protein tyrosine kinases, was inhibited with similar potency (Table 2). Several other protein

Table 2 Concentrations of compounds required for 50% inhibition of the various protein kinases

IC_{50} values were determined from assays carried out at ten different inhibitor concentrations. The ATP concentration was 0.1 mM in all assays.

Compound	Protein Kinase	IC_{50} (μ M)
Roscovitine	CDK2/cyclin A	0.25
	DYRK1A	3.1
	CK1 δ	17
Purvalanol	CDK2/cyclin A	0.1
	DYRK1A	0.3
	RSK1	1.49
Indirubin-3'-monoxime	CDK2/cyclin A	0.59
	GSK3 β	0.19
	AMPK	0.22
	LCK	0.3
	SGK	0.38
Kenpaullone	CDK2/cyclin A	0.67
	GSK3 β	0.23
	LCK	0.47
Alsterpaullone	CDK2/cyclin A	0.08
	GSK3 β	0.11
PP1	LCK	0.47
	LCK	0.05
	CSK	0.52
	SAPK2a/p38	0.64
	CK1 δ	1.06
PP2	LCK	0.06
	CSK	0.73
	SAPK2a/p38	1.43
	CK1 δ	1.29
PP3	CK1 δ	9.9
SP 600125	JNK1 α 1	5.8
	JNK2 α 2	6.1
	PRAK	1
EGCG	PRAK	1
	DYRK1A	0.33

kinases were also inhibited by Kenpaullone (Table 1), but with IC_{50} values of 10 μ M or higher.

Alsterpaullone, a derivative of Kenpaullone, inhibited CDK2/cyclin A and GSK3 β with similar IC_{50} values of 0.08 μ M and 0.11 μ M respectively (Table 2). LCK was inhibited with an IC_{50} value of 0.47 μ M (Table 2). A number of other protein kinases were inhibited less strongly by Alsterpaullone, with IC_{50} values in the range of 5–10 μ M (Table 1).

Inhibitors of Src family kinases

PP1, PP2 and PP3

The related pyrazolopyrimidines, PP1 and PP2, were developed as inhibitors of the Src family of enzymes [17] and have been widely used to suggest physiological roles for these protein tyrosine kinases. For example, the suppression of induction of anti-CD3-induced T-cell activation by PP1 is probably explained by inhibition of the Src family kinases LCK and FYN [17]. However, PP1 and PP2 do not discriminate between the different members of the Src family [17,18].

In our assays, PP1 and PP2 inhibit LCK with IC_{50} values of 50–60 nM, while CSK (a related protein kinase) was inhibited an order of magnitude less potently. In addition, PP1 and PP2 inhibited SAPK2a/p38, SAPK2b/p38 β 2 and CK1 δ with IC_{50} values similar to CSK (Table 2). PP1 and PP2 (10 μ M) also inhibited a number of other protein kinases by 40–60% (Table 1), while PP3, a structurally related molecule that does not inhibit Src family members [18], had little effect on the protein kinases in the panel, apart from CK1 δ (an IC_{50} of 9.9 μ M).

Table 3 Inhibition of protein kinases by commercially available inhibitors

The inhibitor concentrations used are shown in parentheses. Results are presented as kinase activity as a percentage of that in control incubations (means of duplicate determinations). ATP was present at 0.1 mM in all assays. CHK1, checkpoint kinase 1; MAPKAP-K, MAPK-activated protein kinase; MKK1, MAPK kinase 1; PDK1, 3-phosphoinositide-dependent protein kinase 1; PHK, phosphorylase kinase; PKB α , protein kinase B α ; SkMLCK, skeletal muscle MLCK, SmMLCK, smooth muscle MLCK.

Protein Kinase	PP1 (10 μ M)	PP2 (10 μ M)	PP3 (10 μ M)	SU 6656 (10 μ M)	SP 600125 (10 μ M)	KT 5823 (10 μ M)	EGCG (10 μ M)	ML-7 (20 μ M)	ML-9 (100 μ M)
MKK1	52 $\pm$ 4	55 $\pm$ 6	89 $\pm$ 8	82 $\pm$ 3	89 $\pm$ 3	99 $\pm$ 3	94 $\pm$ 3	102 $\pm$ 2	95 $\pm$ 3
MAPK2/ERK2	75 $\pm$ 1	61 $\pm$ 3	92 $\pm$ 6	102 $\pm$ 6	55 $\pm$ 3	106 $\pm$ 3	53 $\pm$ 6	85 $\pm$ 6	101 $\pm$ 3
JNK/SAPK1c	98 $\pm$ 3	98 $\pm$ 1	99 $\pm$ 2	103 $\pm$ 2	38 $\pm$ 5	99 $\pm$ 1	76 $\pm$ 7	97 $\pm$ 3	102 $\pm$ 3
SAPK2a/p38	13 $\pm$ 1	21 $\pm$ 0	100 $\pm$ 5	70 $\pm$ 7	86 $\pm$ 3	102 $\pm$ 4	105 $\pm$ 5	100 $\pm$ 7	94 $\pm$ 1
SAPK2b/p38 β	22 $\pm$ 1	26 $\pm$ 0	84 $\pm$ 2	90 $\pm$ 7	75 $\pm$ 1	100 $\pm$ 5	87 $\pm$ 3	89 $\pm$ 3	98 $\pm$ 1
SAPK3/p38 γ	92 $\pm$ 5	90 $\pm$ 8	83 $\pm$ 2	75 $\pm$ 5	82 $\pm$ 1	103 $\pm$ 1	85 $\pm$ 4	95 $\pm$ 4	98 $\pm$ 2
SAPK4/p38 δ	102 $\pm$ 2	96 $\pm$ 1	95 $\pm$ 2	78 $\pm$ 1	98 $\pm$ 3	105 $\pm$ 6	60 $\pm$ 1	75 $\pm$ 3	86 $\pm$ 3
MAPKAP-K1a	69 $\pm$ 6	114 $\pm$ 2	115 $\pm$ 2	27 $\pm$ 6	46 $\pm$ 3	105 $\pm$ 6	109 $\pm$ 0	99 $\pm$ 2	44 $\pm$ 2
MAPKAP-K2	96 $\pm$ 5	106 $\pm$ 0	98 $\pm$ 2	96 $\pm$ 5	72 $\pm$ 6	71 $\pm$ 5	79 $\pm$ 6	93 $\pm$ 5	106 $\pm$ 7
MSK1	55 $\pm$ 2	57 $\pm$ 3	93 $\pm$ 2	94 $\pm$ 6	51 $\pm$ 7	74 $\pm$ 2	88 $\pm$ 0	59 $\pm$ 1	14 $\pm$ 1
PRAK	85 $\pm$ 7	85 $\pm$ 8	97 $\pm$ 3	95 $\pm$ 2	39 $\pm$ 1	83 $\pm$ 8	10 $\pm$ 2	78 $\pm$ 5	91 $\pm$ 2
PKA	76 $\pm$ 3	74 $\pm$ 3	89 $\pm$ 5	73 $\pm$ 3	82 $\pm$ 1	102 $\pm$ 5	109 $\pm$ 4	85 $\pm$ 1	71 $\pm$ 4
PKC α	80 $\pm$ 3	66 $\pm$ 3	100 $\pm$ 0	90 $\pm$ 4	79 $\pm$ 8	102 $\pm$ 2	76 $\pm$ 1	85 $\pm$ 4	80 $\pm$ 1
PDK1	97 $\pm$ 2	99 $\pm$ 3	82 $\pm$ 3	94 $\pm$ 2	62 $\pm$ 5	114 $\pm$ 5	50 $\pm$ 1	107 $\pm$ 6	113 $\pm$ 4
PKB α	97 $\pm$ 8	77 $\pm$ 7	108 $\pm$ 1	100 $\pm$ 3	95 $\pm$ 2	83 $\pm$ 2	87 $\pm$ 3	88 $\pm$ 2	76 $\pm$ 1
SGK	94 $\pm$ 6	111 $\pm$ 1	94 $\pm$ 2	70 $\pm$ 5	22 $\pm$ 7	72 $\pm$ 5	104 $\pm$ 5	101 $\pm$ 0	84 $\pm$ 4
S6K1	43 $\pm$ 0	70 $\pm$ 3	93 $\pm$ 3	71 $\pm$ 0	22 $\pm$ 4	90 $\pm$ 4	87 $\pm$ 5	74 $\pm$ 1	27 $\pm$ 3
GSK3 β	86 $\pm$ 1	113 $\pm$ 8	99 $\pm$ 0	83 $\pm$ 5	60 $\pm$ 5	52 $\pm$ 1	78 $\pm$ 2	107 $\pm$ 2	107 $\pm$ 5
ROCK-II	65 $\pm$ 4	75 $\pm$ 4	93 $\pm$ 2	43 $\pm$ 6	59 $\pm$ 3	97 $\pm$ 8	53 $\pm$ 7	74 $\pm$ 1	23 $\pm$ 2
AMPK	93 $\pm$ 2	84 $\pm$ 2	94 $\pm$ 2	14 $\pm$ 2	26 $\pm$ 1	90 $\pm$ 2	101 $\pm$ 2	82 $\pm$ 1	61 $\pm$ 1
CHK1	91 $\pm$ 4	93 $\pm$ 1	96 $\pm$ 1	66 $\pm$ 5	39 $\pm$ 0	93 $\pm$ 3	79 $\pm$ 2	94 $\pm$ 2	103 $\pm$ 4
CK2	96 $\pm$ 7	90 $\pm$ 1	76 $\pm$ 7	93 $\pm$ 2	63 $\pm$ 1	98 $\pm$ 5	105 $\pm$ 4	83 $\pm$ 0	69 $\pm$ 1
PHK	70 $\pm$ 6	93 $\pm$ 4	99 $\pm$ 5	21 $\pm$ 1	34 $\pm$ 1	99 $\pm$ 6	78 $\pm$ 8	85 $\pm$ 3	79 $\pm$ 1
LCK	0 $\pm$ 0	1 $\pm$ 0	77 $\pm$ 2	8 $\pm$ 4	53 $\pm$ 1	94 $\pm$ 1	89 $\pm$ 6	87 $\pm$ 1	116 $\pm$ 6
CSK	3 $\pm$ 0	3 $\pm$ 1	96 $\pm$ 7	89 $\pm$ 4	71 $\pm$ 6	93 $\pm$ 1	76 $\pm$ 3	94 $\pm$ 8	96 $\pm$ 4
CDK2/Cyclin A	75 $\pm$ 4	68 $\pm$ 3	84 $\pm$ 0	54 $\pm$ 7	20 $\pm$ 1	94 $\pm$ 4	97 $\pm$ 0	73 $\pm$ 1	75 $\pm$ 1
CK1	8 $\pm$ 1	6 $\pm$ 1	54 $\pm$ 1	66 $\pm$ 5	10 $\pm$ 1	83 $\pm$ 1	105 $\pm$ 7	106 $\pm$ 6	103 $\pm$ 4
DYRK1A	101 $\pm$ 4	94 $\pm$ 4	60 $\pm$ 5	23 $\pm$ 5	16 $\pm$ 6	112 $\pm$ 4	9 $\pm$ 1	66 $\pm$ 8	38 $\pm$ 3
PKG						109 $\pm$ 1			
SmMLCK								10 $\pm$ 1	25 $\pm$ 1
SkMLCK								63 $\pm$ 7	54 $\pm$ 2

SU 6656

The compound SU 6656 is also reported to inhibit members of the Src family of protein kinases [19]. We found that this compound inhibited LCK with an IC₅₀ of 0.04 μ M. Several other protein kinases (e.g. RSK1, AMPK, phosphorylase kinase and DYRK1A) were also inhibited quite strongly (Table 3), but the non-specific effects of SU 6656 differed from PP1 and PP2.

c-Jun N-terminal kinase (JNK) inhibitor SP 600125

The compound SP 600125 was developed as an inhibitor of JNK, a member of the MAPK family [20]. SP 600125 was a relatively weak inhibitor of JNK isoforms, inhibiting JNK1 α 1 and JNK2 α 2 in our assays with IC₅₀ values of 5.8 μ M and 6.1 μ M in our assays at 0.1 mM ATP (Table 2). Moreover, 13 other protein kinases in the panel were inhibited with similar or greater potency by SP 600125. SGK, p70 ribosomal protein S6 kinase (S6K1), AMPK, CDK2, CK1 δ and DYRK1A were all inhibited to a greater extent than JNK (Table 3).

EGCG

This compound is one of the main polyphenolic constituents of tea, especially green tea. We found that EGCG inhibited p38-regulated/activated protein kinase (PRAK; IC₅₀ of 1 μ M) and DYRK1A (IC₅₀ of 0.33 μ M). A few other protein kinases in the panel were inhibited less potently [ERK2, 3-phosphoinositide-dependent protein kinase 1, Rho-dependent protein kinase II

(ROCK-II)], whereas the remainder were unaffected in our assays (Table 2 and Table 3).

KT 5823

KT 5823 is marketed as a highly specific cell-permeable inhibitor of PKG and has been used in many studies to implicate PKG in a variety of cellular processes. These include the activation and proliferation of neuronal [21] and smooth muscle cells [22], as well as endothelin-stimulated migration of neutrophils and monocytes [23]. However, in another report, KT 5823 failed to inhibit an established PKG-mediated response in intact human platelets or rat mesangial cells [24].

KT 5823 (10 μ M) did not inhibit PKG, cAMP-dependent protein kinase (PKA) or protein kinase C (PKC) significantly in our standard assay (Table 3). However, PRAK and GSK3 β were inhibited by approx. 50% at this concentration. It has been noted by others that the potency of KT 5823 towards PKG varies from batch to batch and that some batches are inactive [24]. It is possible that some batches of KT 5823 contain another substance capable of inhibiting PKG.

ML-9 and ML-7

ML-9 was originally reported as an ATP-competitive, cell-permeable inhibitor of myosin light chain kinase (MLCK), PKA and PKC [25]. We found that ML-9 was a relatively weak

inhibitor of smooth and skeletal muscle MLCKs, inhibiting their activities by 75 % and 46 % respectively, at 100 μ M. However, ML-9 also inhibited other protein kinases in the panel, several of which [mitogen- and stress-activated protein kinase 1 (MSK1), S6K1 and ROCK-II] were inhibited more potently than the MLCK isoforms (Table 3).

ML-7, a derivative of ML-9, was reported to be more selective for smooth muscle MLCK than ML-9 [25]. In our assays, ML-7 did indeed prove to be a relatively specific, albeit rather weak, inhibitor of smooth muscle MLCK. MSK1 was also inhibited, but much less potently.

DISCUSSION

In our earlier study [6], we made a number of recommendations for the use of protein-kinase inhibitors in cell-based assays. One of these was that the same effect should be observed with at least two structurally unrelated inhibitors of the protein kinase. The results described in this paper suggest that Kenpaullone should be used in combination with Roscovitine to help identify physiological substrates of CDKs (Table 1). Thus Kenpaullone does not inhibit DYRK1A (which is potently inhibited by Roscovitine), CK1 and RSK1 (which are inhibited less strongly by Roscovitine). Conversely, Roscovitine does not inhibit GSK3 β or LCK (which are potently inhibited by Kenpaullone) or ROCK-II, checkpoint kinase 1 and AMPK (which are inhibited less strongly by Kenpaullone).

Lithium ions inhibit GSK3 [26] and have been shown to mimic cellular effects known to result from the inhibition of GSK3, such as the activation of glycogen synthase [27] and Wingless signalling in *Drosophila* [28]. However, we have reported previously that LiCl also inhibits several other protein kinases with only slightly less potency than GSK3 [6]. Moreover, lithium ions also inhibit inositol monophosphate phosphomonoesterases (reviewed in [29]) and may therefore indirectly affect protein phosphorylation in cells by altering the levels of inositol phosphates. Comparison of the effects of Kenpaullone (Table 1) and LiCl [6] suggests that the combined use of these compounds may help to identify physiological substrates and roles of GSK3. Thus Kenpaullone does not inhibit CK2, MAPK-activated protein kinase 2 and PRAK (Table 1), which are inhibited by LiCl [6], whereas LiCl does not inhibit CDK2, LCK [6] or the other protein kinases that are inhibited by Kenpaullone (Table 1).

Although Alsterpaullone is a more potent inhibitor of GSK3 than Kenpaullone, and Purvalanol is a more potent inhibitor of CDK2 than Roscovitine, our results suggest that Alsterpaullone and Purvalanol are significantly less specific and may therefore be less suitable for use in cell-based assays.

The pyrazolopyrimidines, PP1 and PP2, were developed as inhibitors of the Src family of protein tyrosine kinases [17], explaining their potent inhibition of LCK in our assays (Table 1 and Table 2). However, although relatively selective, PP1 and PP2 also inhibited CSK, SAPK2a/p38, SAPK2b/p38 β 2 and CK1 by approx. one order of magnitude less potently. Since one role of CSK is to inactivate Src family inhibitors via phosphorylation of a C-terminal tyrosine residue [30], the inhibition of CSK may tend to counteract the effect of PP1 and PP2 on Src family members in cells. The inhibition of CSK, SAPK2a/p38 and SAPK2b/p38 β 2 by PP1 and PP2 was not unexpected, because all three protein kinases possess a threonine residue at the position equivalent to Thr¹⁰⁶ of SAPK2a/p38. The small side chain at this position creates a small hydrophobic pocket near the ATP-binding site, the occupancy of which explains the relatively selective inhibition of SAPK2a/p38 and SAPK2b/p38 β 2 by ATP-competitive inhibitors of these enzymes, such as

SB 203580 and SB 202190 [31,32]. Thr¹⁰⁶ is replaced by a bulky hydrophobic residue in most protein kinases, accounting for their insensitivity to SB 203580 and SB 202190. The presence of threonine at the equivalent position in LCK and other Src family members explains why they are also inhibited, albeit less strongly, by SB 203580 and SB 202190 [6]. Conversely, the presence of this particular threonine residue in CSK, SAPK2a/p38 and SAPK2b/p38 β 2 explains their sensitivity to PP1 and PP2, since these compounds occupy the same hydrophobic pocket as SB 203580 [18]. Interestingly, the threonine residue is replaced by an isoleucine residue in the viral oncogenic form of Src (v-Src), explaining why the oncogenic forms of this protein kinase are insensitive to PP1 and PP2 [18].

SP 600125 was developed as an inhibitor of JNK isoforms and reported to show 20-fold selectivity towards a range of protein kinases tested [20]. It was also reported to prevent the expression of several anti-inflammatory genes in cell-based assays [20], the activation of AP1 and expression of collagenase-3 in synoviocytes [33], the expression of type IV collagenase in ovarian cancer (OVCAR) cells [34] and the activation and differentiation of primary human CD4 cell cultures [20]. In animal studies, SP 600125 blocked lipopolysaccharide-induced expression of tumour necrosis factor α and also inhibited anti-CD3-induced apoptosis of CD4⁺ and CD8⁺ thymocytes [20]. For these reasons, JNK was suggested to be an attractive target for the treatment of chronic inflammatory disease, apoptotic cell death and cancer [20]. However, in the present study, we found SP 600125 to be a rather weak inhibitor of JNK isoforms, the lower IC₅₀ values reported previously in [20] being explained by the lower ATP concentrations in the assays. More seriously, SP 600125 was found to be non-specific, and 13 of the 28 protein kinases tested were inhibited with similar or greater potency than JNK. SP 600125 is starting to be used more widely as a JNK inhibitor in cell-based assays [35,36], but our results indicate it is unsuitable for this purpose. The development of more selective JNK inhibitors will be needed to evaluate whether the reported effects of SP 600125 in cells and *in vivo* result from the inhibition of JNK or other enzymes.

Over 1000 papers have been published on the anti-tumour, anti-proliferative, anti-inflammatory and anti-oxidant properties of tea (reviewed in [37]). EGCG, one of the major polyphenolic components of tea, especially green tea, has been reported to inhibit a number of enzymes and signal transduction pathways. For example, it is reported to inhibit activation of the MAPKs ERK1/ERK2, SAPK2a/p38 and SAPK1/JNK in human epidermal keratinocytes after exposure to UV-B radiation and oxidative stress [38], to inhibit the tyrosine phosphorylation of the platelet-derived growth factor receptor in vascular smooth muscle cells [39], to inhibit CDK2 and CDK4 *in vitro* [40] and to increase the expression of the CDK inhibitor p21 in human breast carcinoma cells [40]. EGCG did not inhibit CDK2 in our assays and inhibited only two of the protein kinases in our panel (DYRK1A and PRAK) with IC₅₀ values of 1 μ M or below. PRAK is thought to be activated *in vivo* by SAPK2a/p38. We have found that EGCG not only inhibits the activity of PRAK (Table 1), but also its activation *in vitro* by SAPK2a/p38, whereas the phosphorylation of other proteins by SAPK2a/p38 is unaffected (C. Armstrong and P. Cohen unpublished work). Thus EGCG appears to bind to PRAK in such a way as to prevent SAPK2a/p38 from accessing the threonine residue in the activation loop whose phosphorylation triggers the activation of PRAK.

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REFERENCES

- 1 Cohen, P. (2002) Protein kinases – the major drug targets of the twenty-first century? *Nat. Rev. Drug Disc.* **1**, 309–315
- 2 Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J. and Saltiel, A. R. (1995) A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7686–7689
- 3 Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., Van Dyk, D. E., Pitts, W. J., Earl, R. A., Hobbs, F. et al. (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem.* **273**, 18623–18632
- 4 Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T. and Saltiel, A. R. (1995) PD 98059 is a specific inhibitor of the activation of mitogen-activated protein kinase *in vitro* and *in vivo*. *J. Biol. Chem.* **270**, 27489–27494
- 5 Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P. R. and Lee, J. C. (1995) SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett.* **364**, 229–233
- 6 Davies, S. P., Reddy, H., Caivano, M. and Cohen, P. (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **351**, 95–105
- 7 Brown, E. J., Beal, P. A., Keith, C. T., Chen, J., Shin, T. B. and Schreiber, S. L. (1995) Control of p70 S6 kinase by kinase activity of FRAP *in vivo*. *Nature (London)* **377**, 441–446
- 8 Eysers, P. A., van den Ijssel, P., Quinlan, R. A., Goedert, M. and Cohen, P. (1999) Use of a drug-resistant mutant of stress-activated protein kinase 2a/p38 to validate the *in vivo* specificity of SB 203580. *FEBS Lett.* **451**, 191–196
- 9 Vesely, J., Havlicek, L., Strnad, M., Blow, J. J., Donella-Deana, A., Pinna, L., Letham, D. S., Kato, J., Delvaud, L., Leclerc, S. et al. (1994) Inhibition of cyclin-dependent kinases by purine analogues. *Eur. J. Biochem.* **224**, 771–786
- 10 Meijer, L., Borgne, A., Mulner, O., Chong, J. P., Blow, J. J., Inagaki, N., Inagaki, M., Delcros, J. G. and Moulinoux, J. P. (1997) Biochemical and cellular effects of Roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur. J. Biochem.* **243**, 527–536
- 11 Gray, N. S., Wodicka, L., Thunnissen, A. W. H., Norman, T. C., Kwon, S., Espinoza, F. H., Morgan, D. O., Barnes, G., Leclerc, S., Meijer, L. et al. (1998) Exploiting chemical libraries, structure and genomics in the search for kinase inhibitors. *Science* **281**, 533–538
- 12 Knockaert, M., Lenormand, P., Gray, N., Schultz, P., Pouyssegur, J. and Meijer, L. (2002) p42/p44 MAPKs are intracellular targets of the CDK inhibitor purvalanol. *Oncogene* **21**, 6413–6424
- 13 Hoessel, R., Leclerc, S., Endicott, J. A., Nobel, M. E. M., Lawrie, A., Tunnah, P., Leost, M., Damiens, E., Marie, D., Marko, D. et al. (1999) Iridinibin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent protein kinases. *Nat. Cell Biol.* **1**, 60–67
- 14 Leclerc, S., Garnier, M., Hoessel, R., Marko, D., Bibb, J. A., Snyder, G. L., Greengard, P., Biernat, J., Wu, Y.-Z., Mandelkow, E.-M. et al. (2001) Iridinibin inhibits glycogen synthase kinase-3 β and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* **276**, 251–260
- 15 Schultz, C., Link, A., Leost, M., Zaharevitz, D. W., Gussio, R., Sausville, E. A., Meijer, L. and Kunick, C. (1999) Paullones, a series of cyclin-dependent kinase inhibitors: synthesis, evaluation of CDK1/cyclin B inhibition, and *in vitro* antitumor activity. *J. Med. Chem.* **42**, 2909–2919
- 16 Leost, M., Schultz, C., Link, A., Wu, Y.-Z., Biernat, J., Mandelkow, E.-M., Bibb, J. A., Snyder, G. L., Greengard, P., Zaharevitz, D. W. et al. (2000) Paullones are potent inhibitors of glycogen synthase kinase-3 β and cyclin-dependent kinase 5/p25. *Eur. J. Biochem.* **267**, 5983–5994
- 17 Hanke, J. H., Gardner, J. P., Dow, R. L., Changelian, P. S., Brissette, W. H., Weringer, E. J., Pollok, B. A. and Connelly, P. A. (1996) Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent cell activation. *J. Biol. Chem.* **271**, 695–701
- 18 Liu, Y., Bishop, A., Witucki, L., Kraybill, B., Shimizu, E., Tsien, J., Ubersax, J., Blethrow, J., Morgan, D. O. and Shokat, K. M. (1999) Structural basis for selective inhibition of Src family kinases by PP1. *Chem. Biol.* **6**, 671–678
- 19 Blake, R. A., Broome, M. A., Liu, X., Wu, J., Gishizky, M., Sun, L. and Courtneidge, S. A. (2000) SU 6656, a selective src family kinase inhibitor, used to probe growth factor signaling. *Mol. Cell. Biol.* **20**, 9018–9027
- 20 Bennett, B. L., Saskai, D. T., Murray, B. W., O'Leary, E. C., Sakata, S. T., Xu, W., Leisten, J. C., Motiwala, A., Pierce, S., Satoh, Y. et al. (2001) SP 600125, an anthranyprazolone inhibitor of Jun N-terminal kinase. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 13681–13686
- 21 Firestein, B. L. and Bredt, D. S. (1998) Regulation of sensory neuron precursor proliferation by cyclic GMP-dependent protein kinase. *J. Neurochem.* **71**, 1846–1853
- 22 Chiche, J.-D., Schlutsmeyer, S. M., Bloch, D. B., de la Monte, S. M., Roberts, J., Filippov, G., Janssens, S. P., Rosenzweig, A. and Bloch, K. D. (1998) Adenovirus-mediated gene transfer of cGMP-dependent protein kinase increases the sensitivity of cultured vascular smooth muscle cells to the antiproliferative and pro-apoptotic effects of nitric oxide/cGMP. *J. Biol. Chem.* **273**, 34263–34271
- 23 Elferink, J. G. and De Koster, B. M. (1998) The involvement of protein kinase G in stimulation of neutrophil migration by endothelins. *Eur. J. Pharmacol.* **350**, 285–291
- 24 Burkhardt, M., Glazova, M., Gambaryan, S., Vollkommer, T., Butt, E., Bader, B., Heermeier, K., Lincoln, T. M., Walter, U. and Palmethofer, A. (2000) KT 5823 inhibits cGMP-dependent protein kinase activity *in vitro* but not in intact human platelets and rat mesangial cells. *J. Biol. Chem.* **275**, 33536–33541
- 25 Saitoh, M., Ishikawa, T., Matsushima, S., Naka, M. and Hidaka, H. (1987) Selective inhibition of catalytic activity of smooth muscle myosin light chain kinase. *J. Biol. Chem.* **262**, 7796–7801
- 26 Klein, P. S. and Melton, D. A. (1996) A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8455–8459
- 27 Cheng, K., Creacy, S. and Larner, J. (1983) "Insulin-like" effects of lithium ion on isolated rat adipocytes. II Specific activation of glycogen synthase. *Mol. Cell. Biochem.* **56**, 183–189
- 28 Stambolic, V., Ruel, L. and Woodgett, J. R. (1996) Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr. Biol.* **6**, 1664–1668
- 29 Young, R. C. and Downes, C. P. (1990) Inositol phospholipid-dependent cellular signalling: opportunities for drug discovery. *Drug Des. Delivery* **6**, 1–13
- 30 Nada, S., Okada, M., MacAuley, A., Cooper, J. A. and Nakagawa, H. (1991) Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. *Nature (London)* **351**, 69–72
- 31 Gum, R. J., McLaughlin, M. M., Kumar, S., Wang, Z., Bower, M. J., Lee, J. C., Adama, J. L., Livi, G. P., Goldsmith, E. J. and Young, P. R. (1998) Acquisition of sensitivity of stress-activated protein kinases to the p38 inhibitor, SB 203580, by alteration of one or more amino acids within the ATP binding pocket. *J. Biol. Chem.* **273**, 15605–15610
- 32 Eysers, P. A., Craxton, M., Morrice, N., Cohen, P. and Goedert, M. (1998) Conversion of SB 203580-insensitive MAP kinase family members to drug-sensitive forms by a single amino acid substitution. *Chem. Biol.* **5**, 321–328
- 33 Han, Z., Boyle, D. L., Chang, L., Bennet, B., Karin, M., Yang, L., Manning, A. M. and Firestein, G. S. (2001) c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. *J. Clin. Invest.* **108**, 181–183
- 34 Shin, M., Yan, C. and Boyd, D. (2002) An inhibitor of c-jun aminoterminal kinase (SP 600125) represses c-Jun activation, DNA-binding and PMA-inducible 92 kDa type IV collagenase expression. *Biochim. Biophys. Acta* **1589**, 311–316
- 35 Schnabl, B., Bradham, C. A., Bennett, B. L., Manning, A. M., Stefanovic, B. and Brenner, D. A. (2001) TAK1/JNK and p38 have opposite effects on rat hepatic stellate cells. *Hepatology* **34**, 953–963
- 36 Goss, G. G., Jiang, L., Vondorpe, D. H., Kieller, D., Chernova, M. N., Robertson, M. and Alper, S. L. (2001) Role of JNK in hypertonic activation of Cl⁻-dependent Na⁺/H⁺ exchange in *Xenopus* oocytes. *Am. J. Physiol.* **281**, 1978–1990
- 37 Yang, C. S. and Wang, Z. Y. (1993) Tea and cancer. *J. Natl. Cancer Inst.* **85**, 1038–1049
- 38 Katiyar, S. K., Alaq, F., Azizuddin, K. and Mukhtar, H. (2001) Inhibition of UVB-induced oxidative stress-mediated phosphorylation of mitogen-activated protein kinase signaling pathways in cultured human epidermal keratinocytes by green tea polyphenol (–)-epigallocatechin-3-gallate. *Toxicol. Appl. Pharmacol.* **176**, 110–117
- 39 Ahn, H. Y., Hadizadeh, K. R., Seul, C., Yun, Y. P., Vetter, H. and Sachinidis, A. (1999) Epigallocatechin-3 gallate selectively inhibits the PDGF-BB-induced intracellular signaling transduction pathway in vascular smooth muscle cells and inhibits transformation of sis-transfected NIH 3T3 fibroblasts and human glioblastoma cells (A172). *Mol. Biol. Cell* **10**, 1093–1104
- 40 Liang, Y. C., Lin-Shiau, S. Y., Chen, C. F. and Lin, J. K. (1999) Inhibition of cyclin-dependent kinases 2 and 4 activities as well as induction of cdk inhibitors p21 and p27 during growth arrest of human breast carcinoma cells by (–)-epigallocatechin-3-gallate. *J. Cell. Biochem.* **75**, 1–12