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The selectivity of protein kinase inhibitors; a further update

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Running title: Specificities of protein kinase inhibitors

Abbreviations. The abbreviations for each protein kinase are given in Table S1 in the supplementary section. Other abbreviations;-

AICAR, aminoimidazole-4-carboxamide-1- β -D-ribofuranoside; EGF, epidermal growth factor; FKBP, FK506 binding protein; IGF, insulin like growth factor; IL-1, interleukin 8; NFAT, nuclear factor for activated T-cells; NDRG, N-myc downstream regulated gene; PTEN, phosphatase and tensin homologue.; VEGF, vaso-endothelial-derived growth factor; ZMP, aminoimidazole-4-carboxamide-1- β -D-ribofuranoside monophosphate

Synopsis.

The specificities of 65 compounds reported to be relatively specific inhibitors of protein kinases have been profiled against a panel of 70-80 protein kinases. Based on this information, the effects of compounds that we have studied in cells and other data in the literature, we recommend the use of the following small molecule inhibitors: SB 203580/SB202190 and BIRB 0796 to be used in parallel to assess of the physiological roles of p38 MAPK isoforms, PI-103 and wortmannin to be used in parallel to inhibit PI 3-kinases, PP1 or PP2 to be used in parallel with Src inhibitor-1 to inhibit Src family members; PD 184352 or PD 0325901 to inhibit MKK1 or MKK1 plus MKK5, Akt-I-1/2 to inhibit the activation of PKB/AKT, rapamycin to inhibit TORC1, CT 99021 to inhibit GSK3, BI-D1870 and SL0101 or FMK to be used in parallel to inhibit RSK, D4476 to inhibit CK1, VX680 to inhibit Aurora kinases and roscovitine as a pan CDK inhibitor. We have also identified harmine as a potent and specific inhibitor of DYRK1A in vitro. The results have further emphasised the need for considerable caution in using small molecule inhibitors of protein kinases to assess the physiological roles of these enzymes. Despite being used widely, many of the compounds we analysed were too non-specific for useful conclusions to be made, other than to exclude the involvement of particular protein kinases in cellular processes.

Key words: protein kinase, inhibitor specificity, kinase profiling, drug discovery, anti-cancer drugs.

Introduction.

Small cell-permeant inhibitors of protein kinases have become invaluable reagents with which to investigate the physiological roles of protein kinases, because they can be used simply and rapidly to block endogenous kinase activity in normal cells and tissues, as well as transformed cell lines. In recent years a plethora of protein kinase inhibitors have become available commercially and researchers are often faced with a bewildering variety of compounds from which to choose from, each purported to be a “specific” inhibitor of a particular protein kinase. It is therefore difficult to decide which compound will switch off the activity of the protein kinase or signalling pathway under investigation, both effectively and specifically.

There are some 500 protein kinases encoded by the human genome, most of which are members of the same superfamily, so that the issue of selectivity is critical. Six years ago we studied 28 commonly used protein kinase inhibitors and examined their specificities against a panel of 24 different protein kinases [1], and a few years later extended this analysis to a further 14 compounds against a slightly larger panel [2]. These studies revealed that a number of “specific” inhibitors affected so many protein kinases as to render meaningless the conclusions made about the role of a particular kinase by the use of these compounds. These studies appear to have been useful to the cell signalling community, as judged by the number of times that the first paper [1] was downloaded from the Biochemical Journal website in 2004 (7,600 times) and cited in other papers (over 1,500 times).

Over the past few years we have increased the size of our core “profiling” panel from 30 to over 70 protein kinases and used this enlarged panel to examine further the

specificities of many protein kinase inhibitors. Here, we present information about the specificities of 65 inhibitors and make recommendations about their use. It should be noted that each protein kinase was assayed at or below the K_m for ATP, explaining why the IC_{50} values for some protein kinase inhibitors are lower than those reported in earlier papers where a higher (0.1 mM) concentration of ATP was employed in the assays. These lower concentrations of ATP not only allow a more stringent test of the specificities of protein kinase inhibitors, but also reduce the cost of performing this expensive analysis.

Materials and Methods

Protein kinase inhibitors and other materials.

SB 203580, SB 202190, PP1, PP2, NA-PP1, NM-PP1, SU6656, Src inhibitor-1, ZM 336372, alsterpaullone, kenpaullone, LY 294002, Akt-I-1,2, rapamycin, IC 261, roscovitine, purvalanol, PS 1145, STO 609, SC 514, SP 600125, AS 601245, UCN01, Ro 318220, Go 6976, KT 5720, Rottlerin, H7, H8, H89, HA 1077, H 1152, Y27632 and Compound C were purchased from Calbiochem, GW 5074, SB 216763, SB 415286 and wortmannin from Sigma, harmine, harmalol, harmane and harmaline from Fluka, U0126 from Promega and CK1-7 from Seikegaku Corp, Japan. SL0101 was purchased from Toronto Research Chemicals and one sample was a gift from Morten Frodin, Biotech Research and Innovation Centre, Copenhagen Biocenter, Denmark. LY333531 was a gift from Alex Kozikowski, University of Chicago, USA, BAY439006 a gift from Richard Marais, Institute for Cancer Research, London, UK and FMK a gift from Jack Taunton, UCSF, CA, USA.

BIRB 0796 [3], PD 184352 [4], PD 0325901 and PD 0325901-CI [5], CT 99021[6], BI D1870 [7], AR-A0-14418 [8], PI 103 [9, 10], A-443654[11-13], D4476 [14-16], VX680 [17], BMS-345541[18], CGP 57380 [19], BX 795 and BX 320 [20] and SU6668 [21] were synthesised using the methods indicated. The structures of the compounds that were synthesised are shown in Figure S1 in the supplementary on-line data.

Methylmethane sulphonate was from Sigma, IGF-1 and EGF from Invitrogen, an antibody that recognises the phosphorylated and unphosphorylated forms of ERK5 equally well and phospho-specific antibodies that recognise CHK1 phosphorylated at Ser345, CHK2 at Thr68, PKB at Ser473 and the phosphorylated forms of ERK1/ERK2 were from Cell Signaling Technologies.

Source and purification of kinases.

Unless stated otherwise, all protein kinases were of human origin and encoded full length proteins. Apart from the AMPK complex, which was purified from rat liver, **all other proteins** were either expressed as glutathione S-transferase (GST) fusion proteins in *Escherichia Coli* or as hexahistidine (His6)-tagged proteins in insect Sf21 cells. GST fusion proteins were purified by affinity chromatography on glutathione-Sepharose, and His6-tagged proteins on nickel/nitrilotriacetate-agarose. The procedures for expressing some of the protein kinases used in this paper have been detailed previously [1, 2]. GAK expressed in *E. coli* was a gift from Marjan Ford, MRC Laboratory of Molecular Biology, Cambridge, UK, while IKK α was purchased from Millipore(Upstate). The following sections outline the DNA vectors synthesised and the procedures used to express and purifying protein kinases that have not been reported previously.

Expression of recombinant proteins in E.coli

The following protein kinases were expressed in E.coli:- CHK2[5-543], CK1 δ [1-294], cyclin A2[171-432], cyclin-dependent protein kinase 2 (CDK2), CDK-activating kinase (CAK, also called CDK7) with an additional His₆ tag at its C-terminus, cyclic AMP dependent protein kinase (PKA), phosphorylase kinase [2-297] (PHK[2-297]), calmodulin dependent kinase 1 (CAMK-1), elongation factor 2 kinase (EF2K), c-jun N terminal kinase 3[40-422] (JNK3 α 1[40-422]), the JNK1[M108A] and JNK1[M108G] mutants, MAPK-activated protein kinase-2[46-400] (MAPKAP-K2[46-400] and MAPKAP-K3, smooth muscle myosin light chain kinase [475-838], (smMLCK[475-838]), MAP kinase-interacting kinases 1 and 2 (MNK1, MNK2), PIM2, serine arginine protein kinase 1 (SRPK1), dual specificity tyrosine phosphorylated regulated kinase 1 [1-499] (DYRK1A[1-499]), DYRK2, DYRK3, p21 activated kinases 4, 5 and 6 (PAK4, PAK5 and PAK6), calmodulin dependent kinase kinases α and β (CaMKK α and CaMKK β), maternal embryonic leucine zipper kinase (MELK), extra-cellular signal-regulated kinase 1 (ERK1) and homeodomain-interacting protein kinases 2 [165-564] (HIPK2[165-564]) and HIPK3[161-562]

Expression of recombinant proteins in Sf21 cells

The following protein kinases were expressed in insect Sf21 cells:-p90 ribosomal S6 kinase-1 (RSK1), RSK2, NIMA related protein kinase (NEK) 2a, NEK6[8-313] and NEK7, protein kinase C α (PKC α), Aurora B and Aurora C, extra-cellular signal-regulated kinase 8 (ERK8), I κ B kinase β (IKK β), microtubule affinity regulating kinase 3 (MARK3), mammalian STE-20 like kinase-2 (MST2), protein kinase B α [118-480][S473D], protein kinase B β (PKB β [120-481][S474D], 3-phosphoinositide-

dependent protein kinase-1 [52-556] (PDK1[52-556], Protein kinase D1 (PKD1, also known as PKC μ), Polo like kinase 1 (PLK1), PKC-related kinase 2 [501-984] (PRK2[501-984]), Rho-dependent protein kinase 2 [2-543] (ROCK2[2-543]), serum and glucocorticoid-induced kinase-1 [60-431][S422D] (SGK1[60-431][S422D], (S6K1[1-421][T412E]), Src (chicken), c-Jun N-terminal kinase 2 (JNK2 α 2), PIM1, PIM3, brain-specific kinase 2 (BRSK2), protein kinase C ζ (PKC ζ), mouse lck, c-Raf[306-648][Y340D,Y341D,V492E] (these mutations produce a constitutively active enzyme of high specific activity), B-Raf[2-766][V600E] (the activated oncogenic mutant found in many malignant melanomas), RIP2[2-540] (also called RICK and CARDIAK), IKK ϵ , TBK1, YES, FGF-R1[400-820] and Ephrin A2[591-976].

Activation of protein kinases

In order to produce activated forms of Aurora B and Aurora C, insect Sf21 cells were incubated for 1 h with the protein phosphatase inhibitor okadaic acid (50 nM), while to produce activated PLK1 the Sf21 cells were incubated for 4h with 100 nM okadaic acid prior to harvesting the cells and purifying the enzyme. MKK1 was activated with c-Raf, **wild type and mutant** JNK isoforms with MKK4 and MKK7, p38 MAPK isoforms with MKK6, MAPKAP-K2, MAPKAP-K3, PRAK, MNK1, MNK2 and MSK1 with p38 α MAP kinase, RSK1 and RSK2 with ERK2 plus PDK1; PKB α , PKB β , SGK1 and S6K1 with PDK1, and ERK1 and ERK2 with MKK1. To activate CDK2, bacterial pellets expressing cyclin A2 and CDK2 were mixed together, lyse and purified on glutathione Sepharose. The GST-tags were removed by cleavage with PreScission protease and the CDK2-cyclin A2 complex purified by chromatography on SP-Sepharose. It was then activated with CAK1/CDK7 followed by chromatography on

nickel-nitrilotriacetate agarose to remove CAK1/CDK7, which binds to this column by virtue of its C-terminal His₆ tag. All the other protein kinases were active as expressed.

Protein kinase assays

All assays (25.5 μl) were carried out robotically at room temperature (21 °C) and were linear with respect to time and enzyme concentration under the conditions used. Assays were performed for 30 min using Multidrop Micro reagent dispensers (Thermo Electron Corporation, Waltham, MA 02454, USA) in a 96- well format. The concentration of magnesium acetate in the assays was 10 mM and the [γ -³³P] ATP (800 cpm / pmol) was used at 5, 20 or 50 μM as indicated, in order to be at or below the K_m for ATP for each enzyme. Protein kinases assayed at 5 μM ATP were:- MKK1, ERK1, p38 γ MAPK, p38 δ MAPK, ERK8, PKB α , PKC ζ , PRK2, GSK3 β , CK2, MARK3, IKK β , DYRK3, PIM2, EF2K, PLK1, Aurora C, HIPK2, PAK4. Protein kinases assayed at 20 μM ATP were:- JNK1, JNK2, p38 β MAPK, PDK1, SGK1, S6K1, PKA, ROCK2, PKC α , MSK1, MAPKAP-K2, MAPKAP-K3, PRAK, CaMKK α , CaMKK β , CHK1, CHK2, CDK2, Aurora B, CK1, PIM1, PIM3, NEK7, MST2, HIPK3, PAK5, PAK6, CSK, YES and FGF-R1. Protein kinases assayed at 50 μM ATP were:- Ephrin-A2 receptor (Eph-A2), ERK2, JNK3, p38 α MAPK, RSK1, RSK2, PKB β , PKD1, MNK1, MNK2, AMPK, CaMK1, smMLCK, PHK, BRSK2, MELK, DYRK1a, DYRK2, NEK2a, NEK6, SRPK1, Src, Lck, IKK ϵ and TBK1. Protein kinases assayed at 0.1 mM ATP were RIP2, GAK, c-Raf and B-Raf.

The assays were initiated with MgATP, stopped by addition of 5 μl of 0.5 M orthophosphoric acid and spotted on to P81 filterplates using a unifilter harvester

(PerkinElmer, Boston, MA 02118, USA). The IC₅₀ values of inhibitors were determined after carrying out assays at 10 different concentrations of each compound.

PKA was assayed against the substrate peptide LRRASLG (300 μ M), PKC α and GAK against the protein Histone H1 (0.1 mg/ml for PKC α and 1.0 mg/ml for GAK), PHK against the substrate peptide (KRKQISVRGL) (300 μ M), NEK2a against the peptide RFRRSRRMI (300 μ M), NEK6 and NEK7 against the peptide FLAKSFGSPNRAYKK (300 μ M), ROCK and PRK2 against a peptide corresponding to the C-terminal region of ribosomal protein S6 (KEAKEKRQEQIAKRRRLSSLRASTSKSGGSQK) (30 μ M). Aurora B and Aurora C were both assayed against the substrate peptide LRRLSLGLRRLSLGLRRLSLGLRRLSLG (300 μ M), ERK1, ERK8, HIPK1, HIPK3, MST-2, IKK α and IKK ϵ against myelin basic protein (MBP, 0.33mg/ml), RIP2 against MBP (1.0 mg/ml), IKK β against the peptide LDDRHDSGLDSMKDEEY (300 μ M), and JNK2 and JNK3 against ATF2[19-96] (3 μ M). MARK3 was assayed against the peptide KKKVSRSGLYRSPSPENLNRPR (300 μ M), RSK1, RSK2, MAPKAP-K3 and PKD1 against KKLNRTLVA (30 μ M), MNK1 and MNK2 against the eIF4E protein (0.5mg/ml), EF2K assayed against the peptide RKKFGESKTKTKEFL (300 μ M) and PIM1, PIM2 and PIM3 against RSRHSSYPAGT (300 μ M). PKB β was assayed against the peptide GRPRTSSFAEGKK (30 μ M), PLK1 against ISDELMDATFADQEAKKK (300 μ M), Src against KVEKIGEGTYGVVYK (300 μ M), CAMK-1 against YLRRRLSDSNF (300 μ M), smMLCK against KKRQPQRATSNVFA (300 μ M) and SRPK1 against RSRSRSRSRSRSR (300 μ M). DYRK1A, DYRK2 and DYRK3 were both assayed against Woodtide (KKISGRLSPIMTEQ) (300 μ M), while PAK4, 5 and 6

were assayed against RRRLSFAEPG (300 μ M). CaMKK α , CaMKK β and TBK1 were assayed against (AKPKGKDYHLQTCCGSLAYRRR) (300 μ M), MELK and BRSK2 against (KKLNRTLSFAEPG) (300 μ M), and PKC ζ against (ERMRPRKRQGSVRRV) (300 μ M). The protein tyrosine kinases YES, FGF-R1 and Ephrin A2 were assayed with poly Glu:Tyr (4:1) (1 mg/ml). The substrates used for other protein kinases were described previously[1, 2].

Unless stated otherwise, enzymes were diluted in 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 1 mg/ml BSA, 0.1% (v/v) 2-mercaptoethanol and assayed in 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1% (v/v) 2-mercaptoethanol. For CaMK1 and CaMKK isoforms, the assays also contained 0.5 mM CaCl₂ and 0.3 μ M calmodulin. PKC α was diluted into 20 mM Hepes pH 7.4, 0.03% (v/v) Triton X-100 and assayed in the same buffer containing 0.1 mg/ml phosphatidyl-serine, 10 μ g/ml diacylglycerol and 0.1 mM CaCl₂. PHK (5-20 mU) was diluted in 50 mM Na- β -glycerophosphate pH 7.0, 0.1% (v/v) 2-mercaptoethanol and assayed in 50 mM Tris-HCl-50 mM Na- β -glycerophosphate pH 8.2 and 0.04 mM CaCl₂. EF2K (5-20mU) was diluted into 50 mM Hepes pH 6.6, 0.1% (v/v) 2-mercaptoethanol, 1.0 mg/ml BSA and assayed in the same buffer containing 0.2 mM CaCl₂ and 0.3 μ M calmodulin. smMLCK (5-20mU) was diluted in 50 mM Hepes pH 7.5, 0.1 mM EGTA, 1.0 mg / ml BSA, 0.1% (v/v) 2-mercaptoethanol and assayed in the same buffer containing 5 mM CaCl₂ and 10 μ M calmodulin. PKA (5-20mU) was diluted in 20 mM MOPS pH 7.5, 1 mM EGTA, 0.01% (w/v) Brij35, 1.0 mg/ml BSA, 0.1% (v/v) 2-mercaptoethanol and assayed in 8 mM MOPS pH 7.5, 0.2 mM EDTA. The protein kinases c-Raf and B-Raf were assayed as

described previously [22].

Results and Discussion.

Inhibitors of p38 MAPK (SB 203580, SB 202190, BIRB 0796).

SB 203580 [23] and its close relative SB 202190 have been exploited in thousands of papers to assess the physiological roles of p38 α and p38 β MAPKs. Although these compounds have been, and still are very useful, more recent studies have identified other protein kinases that they inhibit with similar (cyclin G-associated protein kinase (GAK, CK1) or even greater (receptor-interacting protein 2, RIP2) potency [24]. SB 203580 also inhibits c-Raf [25] and GSK3 in vitro (Table 1), albeit less strongly, and inhibits the formation of ZMP, an activator of AMPK, from its inactive precursor AICAR, probably by inhibiting adenosine transporters [26]. Thus there is a danger that the observed effects of SB 203580/SB 202190 on cells result from the inhibition of a target(s) distinct from p38 α /p38 β MAPKs. This inherent problem can be overcome by examining whether the effects of these compounds are no longer observed in cells that express an SB 203580-resistant mutant of p38 α MAPK [27] or p38 β MAPK or by studying whether the results obtained with SB 203580 are also observed in cells from “knockout” mice that do not express p38 α MAPK (e.g.[28]) and/or p38 β MAPK. However, although p38 β MAPK-deficient mice are viable, p38 α MAPK-deficient mice display embryonic lethality, and studies with p38 α MAPK “knockout” cells have so far been confined to the use of embryonic fibroblasts. The availability of inhibitors that are more specific than SB 203580 and SB 202190 would therefore be very useful.

BIRB 0796 is a more potent inhibitor of p38 α and p38 β MAPKs than SB 203580. It interacts with p38 α MAPK in a manner distinct from SB 203580/SB 202190, and its binding induces a slow conformational change that locks the protein into an inactive conformation. Thus the potency of BIRB 0796 increases as the time of pre-incubation with the inhibitor increases [29]. In contrast to SB 203580 or SB 202190, we find that BIRB 0796 does not inhibit CK1 δ , GSK3 β , RIP2 or GAK in vitro (Table 1). However, unlike SB 203580/SB202190, BIRB 0796 also inhibits p38 γ MAPK, p38 δ MAPK and JNK2 α 2 (Table 1). As judged by suppression of the phosphorylation of well established substrates, BIRB 0796 inhibits p38 α MAPK activity completely, when added to the culture medium at only 0.1 μ M, but at 1 μ M it also inhibits p38 γ MAPK. Thus substrates for p38 γ MAPK (and perhaps p38 δ MAPK) can be identified as proteins whose phosphorylation is unaffected at 0.1 μ M BIRB 0796, but inhibited at 1 μ M BIRB 0796 [30]. Although BIRB 0796 is a potent inhibitor of JNK2 in vitro, it does not affect the phosphorylation of JNK substrates (c-Jun, ATF2) in cells at the low (0.1 μ M) concentration that abolishes p38 α MAPK activity in cells, because JNK1 is the dominant isoform that phosphorylates c-Jun and activates the AP1 transcription factor in the cells that have been studied so far [31, 32].

We have used BIRB 0796 extensively to study the role of p38 α MAPK and p38 β MAPK in cell based assays and recommend that it be used in parallel with SB 203580 or SB 202190 when assessing the physiological roles of these protein kinases.

The Src family kinase inhibitors (SU 6656, PP1, PP2 and Src inhibitor 1).

The compound SU 6656 is reported to be a potent inhibitor of Src family members [33]. In the present study we found that it inhibited AMPK, BRSK2 and MST2

with similar potency to Src and Lck, and inhibited Aurora B and C even more potently than Src and Lck in vitro (Tables 1 and 2). SU 6656 also inhibited other protein kinases, such as CaMKK α , CaMKK β , CHK2 and SRPK1 (Table 1). These findings indicate that results obtained by using SU6656 should be interpreted with caution.

The related pyrazolopyrimidines PP1 and PP2 have been used widely to suggest physiological roles for Src family protein kinases, although they do not discriminate between different members of this family [34, 35]. They also inhibit other protein tyrosine kinases, such as the Ephrin receptor EPH A2 and the fibroblast growth factor receptor 1 (FGF-R1) (results not shown). In our assays, PP1 and PP2 inhibited Src and the closely related Lck with IC₅₀ values of 50 nM, while Csk, p38 α MAPK and CK1 δ were inhibited with 3-10 fold lower potency. Interestingly, we found that RIP2 was inhibited even more potently than Src or Lck (Tables 1 and 2) and have recently exploited this finding to identify novel roles for RIP2 in cells [36].

Another compound, termed Src inhibitor 1 (Src-I1), was found to be a potent inhibitor of Src (Table 2), but also inhibited other Src family members, such as Lck, Csk (Table 1) and Yes (results not shown) with similar potency to Src, and RIP2 with even greater potency (Table 2). In addition, it inhibited CHK2 with similar potency to Src, and Aurora B with slightly lower potency (Table 1). However, in contrast to PP1 and PP2 it did not inhibit p38 α /p38 β MAPKs or CK1 δ (Table 1). We therefore recommend that PP1 or PP2 be used in parallel with Src inhibitor 1 to assess the physiological roles of the Src family of protein tyrosine kinases.

The PP1 derivatives NM-PP1 and NA-PP1.

A significant subset of protein kinases, including Src, Lck, p38 α /p38 β MAPKs, GAK, RIP2 and a number of receptor tyrosine kinases, possess a threonine residue at the so-called “gatekeeper” site. This creates a hydrophobic pocket near the ATP-binding site, which underlies the sensitivity of these enzymes to compounds such as PP1/PP2 and/or SB 203580. In contrast, these compounds do not inhibit most protein kinases because they possess a bulky hydrophobic residue at this position. For example in v-Src, the virally encoded form of Src, the threonine residue is replaced by isoleucine, explaining why this oncogene is insensitive to PP1/PP2 [35]. However, by mutating the residue at the gatekeeper site to threonine or other amino acids with even smaller side chains (Ser, Ala and Gly), it is possible to convert protein kinases to forms that can be potently inhibited by PP1, PP2 or SB 203580. Conversely, the mutation of the “gatekeeper” threonine to an amino acid with a larger side chain converts these protein kinases to SB203580-insensitive forms [37-39].

Recently, “knock-in” mice have been generated that express a mutated form of JNK in which the gatekeeper methionine has been changed to glycine [31, 32]. In contrast to wild-type JNK, the mutated JNK can be inhibited by modified PP1 derivatives, such as NA-PP1 and NM-PP1. Potentially, this is a powerful way of studying the physiological roles of protein kinases, because the mutated kinase possesses similar activity to the wild-type enzyme, but can be inhibited rapidly and reversibly by adding NA-PP1 or NM-PP1 to the culture medium. However, the general applicability of this approach depends, in part, on the selectivity with which NA-PP1 and NM-PP1 inhibit the mutant protein kinases, compared to the other wild type protein kinases that

are expressed endogenously in the same cells and tissues. We therefore examined the specificities of NA-PP1 and NM-PP1 against our extended panel.

The specificities of NA-PP1 and NM-PP1 were similar to PP1 and PP2, these compounds inhibiting RIP2, GAK, CK1 and p38 α / β MAPK, as well as Src, Lck and Csk (Table 1) and other protein tyrosine kinases like the EPH-A2 and FGF-R1 receptors (results not shown). Additionally, we found that NA-PP1 and NM-PP1 inhibited PKD1, and MST2, while NM-PP1 also inhibits PKA (Tables 1 and 2). We also found that the concentrations of NA-PP1 and NM-PP1 required to inhibit the “gatekeeper” mutants of JNK1 (JNK1[M108A] and JNK1[M108G]) were similar to those required to inhibit Src family kinases, RIP2 and PKD (Table 2). Wild type JNK1 was not inhibited by NA-PP1 or NM-PP1 (Table 1).

These findings suggest that caution may be needed in interpreting experiments performed using cells and tissues from mice that express the “gatekeeper” mutants of protein kinases (sensitised to inhibition by NA-PP1/NM-PP1) instead of the wild type enzymes. Although control experiments can be carried out using cells/tissues from wild type or knock-out mice that do not express the protein kinase, to check for “off-target” effects of NA-PP1 and NM-PP1, it is often necessary to inhibit protein kinases in two different signalling pathways in order to suppress the phosphorylation of a particular protein or biological process. For example, the combined inhibition of MKK1 and p38 α MAPK is needed to suppress the phosphorylation of CREB induced by EGF or UV-C radiation [40, 41], while the combined inhibition of PI 3-kinase and MKK1 is needed to prevent the EGF stimulated phosphorylation of GSK3 [41]. It is therefore possible that the effects of NA-PP1/NM-PP1 on cells do not always result from the inhibition of the

“gatekeeper” mutant kinase alone, but may result from the combined inhibition of the mutant kinase and one or more other intracellular protein kinases, such as Src family members, RIP2 and PKD1, which are inhibited by these compounds at similar concentrations.

The Raf inhibitors ZM 336372, BAY439006 and GW 5074.

The Raf isoforms lie at the head of the classical growth factor-stimulated MAP kinase cascade that plays a key role in stimulating cells to proliferate or differentiate. Activating mutations in B-Raf occur in many cancers, and with high frequency in malignant melanoma. ZM 336372 was originally developed as a c-Raf inhibitor. Like p38 α MAPK and p38 β MAPK, Raf possesses a threonine residue at the “gatekeeper” site, explaining why ZM 336372 inhibits p38 α/β MAPKs and why SB 203580 inhibits Raf. Thus the mutation of Thr106 in p38 α MAPK to Met makes it insensitive to both ZM 336372 and SB 203580 [25, 42]. Here, we extended the specificity of ZM 336372 to 70 protein kinases, which established that it does not inhibit other protein kinases tested significantly apart from three that possess a threonine residue at the “gatekeeper” site (p38 α MAPK, p38 β MAPK and Lck) (Tables 1 and 2).

Despite being a potent and specific inhibitor of Raf, ZM 336372 does not prevent the growth factor or phorbol-ester-induced activation of MKK1 or ERK1/ERK2 and, unlike inhibitors of MKK1, it does not reverse the phenotype of Ras or Raf-transformed cell lines [42]. This appears to be explained by a feedback control loop in which Raf efficiently prevents its own activation, such that the inhibition of Raf by ZM 336372 is always counterbalanced by an equivalent activation, resulting from the suppression of

this feedback loop [42]. These findings have highlighted a problem in targeting Raf for the development of anti-cancer drugs.

BAY 439006 was also developed initially as a Raf inhibitor [43] and, in the present study, we found that its specificity resembles that of ZM 336372. Thus, like ZM 336372, BAY 439006 also inhibits p38 α MAPK, p38 β MAPK, Src and Lck. However, unlike ZM 336372, BAY 439006 also inhibits RIP2, Aurora kinases, HIPK2, HIPK3 and ERK8 (Tables 1 and 2). BAY 439006 (also called Nexavar) has been approved for the treatment of kidney cancer and gastrointestinal tumours that are resistant to Gleevec. Although originally believed to exert its anti-cancer effects by inhibiting Raf, more recent studies have demonstrated that it also inhibits a number of receptor tyrosine kinases that possess threonine at the “gatekeeper” site, and that inhibition of these targets, rather than Raf, is likely to underlie its clinical efficacy [44]. Consistent with this we have found that BAY 439006 inhibits the FGFR1 and the EPH-A2 receptor tyrosine kinases (results not shown).

GW 5074 is another inhibitor of Raf isoforms (Table 2). In the present study we found that this compound inhibited the three PIM isoforms more potently than Raf, and it also inhibited several other protein kinases, such as HIPK2, RIP2, GAK and MST2 with comparable potency to Raf (Tables 1 and 2).

In summary, the feedback control mechanism by which Raf suppresses its own activation means that no compounds have yet been developed that convincingly prevent the activation of the classical MAP kinase cascade by inhibiting Raf, and inhibitors of MKK1 are being used for this purpose instead, as detailed below.

Inhibitors of MKK1 and its activation (U0126, PD184352, PD 0325901 and PD 0325901-CI).

MKK1 (also called MEK1) is activated by Raf *in vivo* and is also being targeted to develop anti-cancer drugs. PD 98059 [45] and U0126 [46] were the first compounds to be described that target MKK1 and the closely related MKK2, and have been exploited in thousands of subsequent papers. Although initially identified by their ability to inhibit a mutated form of MKK1 that possesses some constitutive activity *in vitro*, PD 98059 and U0126 are non-competitive inhibitors that appear to interact with the inactive unphosphorylated kinase more strongly than the active phosphorylated species and therefore exert their effects in cell based assays by preventing the phosphorylation of MKK1 and/or the conformational transition that generates the activated enzyme [1, 45]. More recently, additional non-competitive inhibitors of MKK1 with greater potency (PD 184352 [47] and PD 0325901 [48]) have been developed and have entered clinical trials as anti-cancer agents.

The specificities of U0126, PD 184352, PD 0325901 and the S-stereoisomer of a closely related compound, termed here PD 0325901-CI (Fig S1 in the supplementary on-line section), are compared in Table 3. PD 184352, PD 0325901 and PD 0325901-CI inhibited the active phosphorylated form of MKK1 with IC_{50} values close to 1 μ M *in vitro*, while U0126 inhibited activated MKK1 with about 10-fold lower potency. However, these non-competitive inhibitors suppressed the activation of ERK1/ERK2 (the substrates of MKK1) at much lower concentrations in cell based assays, presumably because they bind even more strongly to the inactive unphosphorylated form of MKK1. We have reported previously, that the EGF-induced activation of ERK1/ERK2 was

completely suppressed at 10 μM U0126 or 1 μM PD 184352 in Swiss 3T3 cells [1]. In the present study, we found that PD 0325901 and the S and R isomers of PD 0325901-Cl were even more potent inhibitors than PD 184352. PD 0325901 and the S isomer of PD 0325901-Cl suppressed the activation of ERK1/ERK2 at 25 nM in EGF-stimulated HeLa cells, as compared to 0.5 μM for PD 184352 in parallel experiments. The R-isomer of PD 0325901-Cl was a slightly less potent inhibitor than the S-isomer (Fig 1A). At these concentrations, no other protein kinases in our panel were inhibited and, even at 10 μM , only a few protein kinases were inhibited slightly (Table 3).

PD 98059 and U0126 have been reported to inhibit MKK5, a protein kinase closely related to MKK1, with similar potency to MKK1 [49]. Thus these compounds also prevent the activation of ERK5, the physiological substrate of MKK5. We have reported that concentrations of PD 184352 which block the activation of ERK1/ERK2 in cells (1-2 μM) do not affect the activation of ERK5, and that higher concentrations (10-20 μM) are needed to prevent the activation of ERK5 in cells [50]. Here we show that PD 0325901 and PD 0325901-Cl also prevent the activation of ERK1/ERK2 in cells at concentrations that do not affect the activation of ERK5, as judged by their failure to prevent the EGF-induced phosphorylation of ERK5, measured by a decrease in electrophoretic mobility. However, these compounds blocked the activation of ERK5 when included in the culture medium at concentrations of 2 μM or higher (Fig 1B).

In summary, PD 184352 and PD 0325901/PD 0325901-Cl are both extremely potent and selective inhibitors of MKK1 (and the closely related MKK2) in cell-based assays and can also be used to suppress the activation of ERK5. Physiological substrates for ERK5 can be identified as proteins whose phosphorylation in cells is unaffected by

0.1 μM PD 0325901 but prevented by 2 μM PD 0325901, or as proteins whose phosphorylation is unaffected by 1-2 μM PD 184352 but suppressed 10-20 μM PD 184352. We recommend that PD 184352 or PD 0325901 be used to inhibit MKK1 in cells. The structurally unrelated U0126 can be used to check the results.

The RSK inhibitors BI-D1870, SL0101 and FMK.

The RSK isoforms are activated by ERK1/ERK2 and are the most downstream kinases of the classical MAP kinase cascade. We have recently described BI-D1870 as a relatively specific nanomolar inhibitor of RSK isoforms and exploited it to identify physiological substrates and roles for RSK in cells [51]. BI-D1870 was originally developed on a programme to identify inhibitors of Polo-like kinases (PLKs) and it also inhibits PLK1 with slightly lower potency than RSK isoforms, while Aurora B, MELK, PIM3 and MST2, were inhibited with 10 to 100-fold lower potency and other protein kinases tested were unaffected [51] (Table 3).

In the present study we compared BI-D1870 with SL0101 [52] and FMK [53], two other recently described inhibitors of RSK (Table 3). These experiments revealed that SL0101 was also a relatively specific inhibitor of RSK isoforms, but much less potent than BI-D1870 (Table 1). SL0101 inhibited Aurora B, PIM1 and PIM3 with slightly lower potency than RSK1/RSK2, but other protein kinases in the panel were unaffected, including PLK1.

RSK isoforms are unusual in possessing two protein kinase domains in the same polypeptide. ERK1/ERK2 phosphorylate and activate the C-terminal kinase domain,

which then activates the N-terminal kinase domain, enabling the N-terminal kinase domain to phosphorylate other proteins. FMK is an irreversible inhibitor that covalently modifies the C-terminal kinase domain of RSK. It therefore prevents the activation of the N-terminal kinase domain of RSK by the C-terminal kinase domain, but does not affect the activity of the N-terminal domain, explaining why the active forms of RSK1 and RSK2 were not inhibited by FMK in vitro (Table 3 and [53]). This contrasts with BI-D1870 and SL0101, which inhibit the N-terminal kinase domain. In the present study we found that FMK inhibited relatively few protein kinases in the panel, although it did inhibit the protein tyrosine kinases, such as Src, Lck and Yes and the EPH-A2 receptor, as well as S6K1 (Tables 3 and 5).

In summary, we [51] and others [54] have found D1870 to be a useful inhibitor of RSK isoforms in cells and recommend it for this purpose, although it should be born in mind that Polo-like kinases will also be inhibited. SL0101 [52] (Colin Watts personal communication) and FMK [55] are also useful. FMK is the only known inhibitor of the C-terminal kinase domain of RSK and may therefore have a further use in preventing the phosphorylation of any proteins, besides the N-terminal kinase domain of RSK, that might be targeted by the C-terminal domain in cells. However, FMK would not inhibit RSK if the N-terminal kinase domain was activated by a mechanism that was independent of the C-terminal domain, as has recently been observed [54, 55].

Inhibitors of GSK3 (CT 99021, AR-A0-144-18, SB 216763, SB 415286, alsterpaullone, kenpaullone, and LiCl).

Inhibitors of GSK3 are being developed as potential drugs to treat diabetes, stroke, Alzheimer's and other diseases [56]. The compounds alsterpaullone, kenpaullone

[2], CT 99021, AR-A0144-18, SB 216763 and SB 415286 [57] inhibit GSK3 at nanomolar concentrations. Here, we found that CT 99021 was the most potent and specific inhibitor in vitro. It inhibited CDK2-cyclin A about 50-fold less potently and did not affect other protein kinases in the panel significantly at 1 μ M (Tables 2 and 3). Apart from AR-A014418, the other four GSK3 inhibitors inhibited several other protein kinases in addition to CDK2-cyclin A. For example, SB 216763 inhibited ERK8, DYRK1A, PIM3, SRPK1 and HIPK2, SB 415286 inhibited MKK1, ERK8 and MST2 and several other protein kinases to a slightly lesser extent, kenpaullone inhibited PHK, CHK2, PAK4, PAK5, PIM3, Src and Lck, while alsterpaullone inhibited PHK and CHK2 (Table 3). SB 216763, SB 415286, kenpaullone and alsterpaullone also inhibited other protein kinases less strongly.

Lithium ions inhibit GSK3 in the millimolar range and its effects in cell-based assays have been used to suggest physiological roles for this enzyme. In the present study we found that LiCl inhibited GSK3 β activity in vitro more strongly than any of the other protein kinases tested. However, LiCl inhibited a number of other protein kinases with slightly lower potency than GSK3, including, MNK1, MNK2, smooth muscle MLCK, PHK, CHK2, HIPK3, IKK ϵ and TBK1 (Table 3).

In summary, we recommend using CT 99021 to inhibit GSK3 in cells, as it is the most potent and specific inhibitor available. When added to the cell culture medium at 1-2 μ M, it completely prevents the phosphorylation of authentic GSK3 substrates such as N-myc downstream-regulated gene 1 (NDRG1) [57] and c-Jun at Thr239 (S.Morton and P.Cohen, unpublished work). Results obtained with CT 99021 can be checked by using one or more of the other GSK3 inhibitors.

Inhibitors of the PtdIns 3-kinase superfamily (wortmannin, LY 294002, PI 103 and rapamycin).

Many cancers are caused by activating mutations in phosphatidylinositol (PI) 3-kinase α or inhibitory mutations in PTEN, the phosphatase that reconverts PI(3,4,5)P₃ (the product of the PI 3-kinase reaction) to PI(4,5)P₂. For this reason, the development of potent and specific inhibitors of Class 1 PtdIns 3-kinases has recently become of great interest for the development of novel anti-cancer drugs.

The fungal metabolite wortmannin was originally known as a potent inhibitor of the neutrophil respiratory burst and was shown subsequently to inhibit smooth muscle myosin light chain kinase (smMLCK) [58]. However, it later became clear that it was a far more potent inhibitor of Class 1 and Class 2 phosphatidylinositol (PI) 3-kinases than MLCK, and completely suppresses their activities when added to the cell culture medium at only 50-100 nM. More recently, wortmannin was also found to inhibit Polo-like kinase 1 (PLK1) [59]. We therefore re-examined its specificity against our extended panel. These studies confirmed that wortmannin inhibited smMLCK and PLK1 in our assays in the micromolar range (Tables 4 and 5), but no other protein kinases in the panel were inhibited significantly. At micromolar concentrations, wortmannin is also reported to inhibit a PI 4-kinase and the mammalian target of rapamycin (mTOR), another member of the PI 3-kinase superfamily.

LY 294002 is another commonly used, but less potent inhibitor of PI 3-kinases, which inhibits Class 1 PI 3-kinases at 10-50 μ M in cell-based assays. It has been the inhibitor of choice when cells are incubated for prolonged periods, because wortmannin is unstable in aqueous solution. However, LY 294002 is also reported to inhibit other

kinases, such as TORC1, CK2 [1] and PLK1 [59] at concentrations similar to those that inhibit PI 3-kinases [1]. Using our extended panel, we now find that LY 294002 also inhibits PIM1, PIM3, HIPK2 and GSK3 (Tables 2 and 4), again at concentrations similar to those that inhibit Class 1 PI 3-kinases. Immobilised LY 294002 was recently shown to bind GSK3 and a number of other ATP-binding proteins that are not protein kinases [60].

In cell-based assays PI-103 [61] blocks Class 1 PI 3-kinases completely at only 0.5 μ M, as judged by suppression of the activation of PKB (Fig 2A). However, it inhibited relatively few of the 70 protein kinases in our panel and then by no more than 30-40%, even when assayed in vitro at 1 μ M and at low ATP concentrations (Table 4). Moreover, PI 103 at this concentrations did not affect two other members of the PI 3-kinase superfamily, the protein kinases ATM and ATR, as judged by its failure to suppress the phosphorylation (activation) of their substrates, the protein kinases CHK1 and CHK2, in cell-based assays (Fig 2B). However, in another recent study, PI 103 was shown to inhibit TORC1 with similar potency to Class 1 PI 3-kinases [62].

Rapamycin is a naturally occurring compound produced by the soil bacterium *Streptomyces hygroscopicus* that originates from Easter Island (Rapa Nui is the native name for Easter Island). It was first purified over 35 years ago as an anti-fungal agent, but was originally discarded because of its undesirable immunosuppressive side effects. Its potential as an immunosuppressive drug was only explored many years later and it was finally approved as an immunosuppressant in 1999. It is used most frequently to prevent tissue rejection after kidney and pancreatic islet transplantation. The anti-cancer properties of rapamycin were also noticed in the mid 1970's and a modified form of rapamycin have recently been approved for clinical use. Rapamycin exerts its effects on

cells by binding to the FK506 binding protein (FKBP) and the molecular target for the rapamycin-FKBP complex was identified as the Target Of Rapamycin Complex 1 (TORC1) [63]. The unusual mechanism of action of rapamycin may explain why it does not inhibit any protein kinase in our extended panel (Table 4), or any other protein kinase that has been tested, even at a concentration of 1 μ M, which is 10-20-fold higher than that required to inhibit TORC1 activity completely in cell-based assays.

In summary, while wortmannin continues to be very useful as an inhibitor of PI 3-kinases in cell-based assays, we recommend that the use of LY 294002 be discontinued and that it be replaced by PI-103. Rapamycin is an exquisitely specific inhibitor of TORC1 and should be used in parallel to check whether any of the observed effects of PI-103 result from the inhibition of TORC1, rather than PI 3-kinases.

The PDK1 inhibitors BX 795 and BX 320.

PDK1 catalyses the activation of PKB isoforms, a reaction that requires the presence PtdIns (3,4,5)P₃, the product of the PI 3-kinase catalysed reaction. Mice expressing 15% of the normal level of PDK1 are strikingly protected against the formation of multiple tumours that occur in animals carrying only one copy of the PTEN gene [64]. For this reason, PDK1 has become an attractive target for an anti-cancer drug [65]. BX 795 and BX 320 have been described as potent and specific inhibitors of PDK1 [66] and are beginning to be used to block its activity in cells. In the present study we found that BX 795 was not only a potent inhibitor of PDK1, but also inhibited ERK8, MNK2, Aurora B, Aurora C, MARK3 and IKK ϵ with similar potency. TBK1 was inhibited even more potently than PDK1 (Table 4). The IC₅₀ values for inhibition of these protein kinases in our assays were:- PDK1 (17 nM), Aurora B (11 nM), IKK ϵ (9.5

nM) and TBK1 (2.3 nM). The specificity of BX 320 was similar to BX 795, although it was a much less potent inhibitor.

Interestingly, Aurora kinase (see below) and TBK1 [67, 68], like PDK1, are also attractive targets for the development of anti-cancer drugs. TBK1 is activated in response to hypoxia [67] and controls the production of angiogenic factors, such as VEGF and IL-8. Moreover, its levels are elevated in malignant colon and breast cancer cells. TBK1 is also reported to be activated by the RalB/Sec5 effector complex, restricting the initiation of apoptotic programmes and so aiding tumour cell survival [68]. BX795 and other compounds that are potent inhibitors of these three protein kinases might therefore be particularly effective as anti-cancer agents.

The present study indicates that BX 320 and BX 795 are not specific inhibitors of PDK1, but might be useful for assessing the physiological roles of TBK1 and the closely-related IKK ϵ , as they are the most potent inhibitors of these two protein kinases to be described thus far.

The PKB inhibitors A-443654 and Akt-I-1,2.

PKB (also called AKT), a protein kinase that is activated by PDK1 *in vivo*, has also attracted considerable interest as an anti-cancer target. A-443654 has been described as a specific inhibitor of PKB [69], and is being used to ascribe particular functions to this protein kinase. In the present study we confirmed that this compound was indeed a very potent inhibitor of PKB, but found that it also inhibits some other members of the AGC subfamily of protein kinases with slightly lower potency, such as PKA, PRK2 and MSK1, and it also inhibited DYRK1A. Several other protein kinases were inhibited to a

lesser extent (Table 4). These analyses show that A-443654 is not a selective PKB inhibitor and should be used with considerable caution.

In contrast to A-443654, Akt-I-1,2 is a highly selective non-competitive inhibitor of PKB *in vitro* [70]. At a concentration of 1 μ M, it inhibits full length PKB α /AKT1 or CAMK1 by 80%, but no other protein kinase in the panel, including the catalytic domains of PKB α and PKB β , was inhibited significantly at this concentration (Table 4). This is because inhibition by Akt-I-1,2 requires the presence of the pleckstrin homology (PH) domain. Importantly, Akt-I-1,2 prevents the conformational change, triggered by the binding of PI(3,4,5)P₃ to the PH domains of PKB isoforms, that allows PDK1 and TORC2 to phosphorylate and activate PKB. For this reason, Akt-I-1,2 is a potent inhibitor of the activation of PKB than of active PKB, and prevents the insulin-induced activation of PKB/AKT when added to cells at 1 μ M [71].

In summary, we recommend the use of Akt-I-1,2 to inhibit PKB in cells.

Inhibitors of CK1 (D4476, CK1-7 and IC 261).

CK1 isoforms play multiple roles in cell regulation. We have previously reported that the compound D4476 synthesised during a programme to develop inhibitors of the Activin Receptor-like Kinase 5 (ALK5), was a relatively selective inhibitor of CK1, and more potent than the other known the CK1 inhibitors CK1-7 and IC261 against 30 protein kinases [72]. Here, we extended these studies to the larger panel (Tables 5 and 6). The results confirmed that D4476 is a rather selective inhibitor of CK1. D4476 inhibited CK1 20-30-fold more potently than PKD1 or p38 α MAPK and no other protein kinases in the panel were inhibited to a significant extent. CK1-7 and IC261 were 5-10 fold less potent inhibitors of CK1 and also inhibited several other protein

kinases, including PIM1 and PIM3 (CK1-7 and IC261), ERK8, MNK1, AMPK, SGK1 (CK1-7) (Table 6). We recommend the use of D4476 to inhibit CK1 isoforms in cell-based assays. A method for preventing its precipitation in aqueous solution has been described [72].

Identification of harmine as a specific inhibitor of DYRK isoforms.

Healers in the Amazon have been using harmine as a psychoactive compound in a brew known as “ayahuasca” for thousands of years. A serotonin antagonist and reversible short-term inhibitor of monoamine oxidase, it was first used to treat Parkinsonism in 1928, where it was said to brighten the mental status of the patients. However, it is also of interest as an anti-cancer agent and, in this connection, was reported to inhibit cyclin-dependent protein kinases in the micromolar range [73]. These findings led us to examine its specificity against our panel of protein kinases, which revealed that harmine was an extremely potent and specific inhibitor of the DYRK family of protein kinases (Table 6). It inhibited DYRK1A in the nanomolar range, the DYRK2 and DYRK3 isoforms being inhibited about 10-fold less potently. In our experiments harmine did not inhibit CDK2 significantly, but did inhibit the three PIM isoforms and CK1 in the micromolar range (Tables 5 and 6).

Down’s syndrome resulting from the presence of an extra copy of chromosome 21 is the most common genetic disorder in humans with a frequency of 1 in 800 live births. The Down’s syndrome child begins life with an IQ close to that of a normal child but these parameters gradually deteriorate, until at aged 13 they display an average IQ of 50. Interestingly, the gene encoding DYRK1A is located within the Down’s Syndrome Critical Region. DYRK1A is expressed at elevated levels in human Down’s syndrome

foetal tissues, and mice that over-express this kinase have defects in neural development [74]. A recent report suggested that the pathological effects of high DYRK1A activity may result from the hyper-phosphorylation and reduced activity of the transcription factor NFATc [75]. The finding that harmine is a potent and specific inhibitor of DYRK1A raises the possibility of preventing mental retardation in Down's syndrome patients through the use of drugs, such as harmine or a derivative of this compound, that inhibit this protein kinase.

Very recently, harmine was identified as an anti-diabetic, cell-type-specific regulator of PPAR γ expression and, when administered to diabetic mice, it mimicked the effect of PPAR γ ligands on adipocyte gene expression and sensitivity to insulin [76]. It will clearly be of great interest to find out whether the anti-diabetic effects of harmine are explained by its ability to inhibit one or more DYRK isoforms.

The potent inhibition of DYRK1A by harmine was unexpected given its rather low molecular mass (212 Da) and understanding how this drug interacts with DYRK1A will be of considerable interest. The related compounds, harmalol, harmaline and harmine were also relatively specific, but much weaker inhibitors of DYRK isoforms (Table 6). However, currently, there is no information as to whether harmine can suppress the activity of DYRK1A in cells.

The CDK inhibitors Roscovitine and Purvalanol A.

The olomoucine derivatives, roscovitine [77] and purvalanol [78] were identified as cyclin-dependent protein kinase (CDK) inhibitors a number of years ago. Purvalanol was found to inhibit several protein kinases in our panel, such as PAK4, PAK5, MELK, Src, (Tables 5 and 7) and Yes (results not shown), although not as potently as CDK2

Roscovitine inhibited ERK8 but was only a weak inhibitor of other protein kinases (Tables 5 and 7). Roscovitine and purvalanol are known to inhibit other CDKs with similar potency to CDK2, including CDK1, CDK5 and CDK7 [77, 78], while Roscovitine also inhibits pyridoxal kinase [79]. These findings support the continued use of these two compounds as pan CDK inhibitors.

The Aurora kinase inhibitor VX 680 and SU6668.

VX 680 was developed as a potent inhibitor of Aurora kinases, which regulate several aspects of the cell division cycle, including microtubule-kinetochore attachments. For this reason Aurora kinases are being targeted for the development of anti-cancer drugs and some have entered clinical trials. More recently, VX 680 was also found to be a potent inhibitor of the ABL protein tyrosine kinase [80]. We found that VX 680 also inhibited MELK and Src and other protein kinases, such as FGF-R1 and Eph-A2 (results not shown), with less marked inhibition of several other protein kinases such as ERK8, RSK1, RSK2, PAK4 and MST2 (Tables 5 and 7). VX680 has also been reported to inhibit the protein tyrosine kinase FLT3, although not as potently as Aurora kinases [81]. VX 680 appears to inactivate Aurora A and Aurora B completely when added to the cell culture medium at 1 μ M, as judged by the blockade of Transforming Acidic Coiled Coil Protein 3 (an Aurora A substrate) and histone H3 (an Aurora B substrate) [64].

SU 6668 was developed to inhibit the vasoendothelial-derived growth factor (VEGF) receptor and fibroblast growth factor (FGF) receptor with the aim of inhibiting tumour growth by suppressing angiogenesis, but it has recently been found to bind to and inhibit several other protein kinases, including Aurora kinases, TBK1 and AMPK [82].

When profiled against our extended panel, we found that SU6668 not only inhibited these

protein kinases, but a number of others. MKK1, CHK2, ERK8, RSK1, RSK2, S6K1, Aurora B and Aurora C were the protein kinases inhibited most potently (Tables 5 and 7). These findings indicate that SU 6668 is insufficiently specific to be useful as a protein kinase inhibitor in cell based assays.

The calmodulin-dependent protein kinase kinase inhibitor STO 609.

STO 609 has been identified as an inhibitor of calmodulin-dependent protein kinase kinases α and β , which are “upstream” activators of calmodulin-dependent protein kinases 1 and 4. CaMKK β also activates the AMP-activated protein kinase (AMPK) in neuronal cells [83] and T-cells [84]. When tested against our extended panel, CaMKK β was inhibited about 10-fold more potently than CaMKK α . However, STO609 was also inhibited ERK8, MNK1, CK2, AMPK PIM2, PIM3, DYRK2, DYRK3 and HIPK2 with similar potency to CaMKK α (Tables 5 and 7). STO609 suppresses CaMKK activity almost completely when added to cells at 25 μ M (Grahame Hardie, personal communication). However, although this compound has been used to implicate CaMKKs in the activation of AMPK, the present study indicates that STO609 is not a specific inhibitor and results obtained by using it should be interpreted with caution.

An inhibitor of AMPK (Compound C).

This compound has been described as an inhibitor of AMPK and is being used increasingly to inhibit this protein kinase in cell-based assays. In the present study we found that Compound C inhibited AMPK with an IC₅₀ value of 0.1-0.2 μ M, but a number of other protein kinases were inhibited with similar or greater potency, including ERK8, MNK1, PHK, MELK, DYRK isoforms, HIPK2, Src, Lck (Table 7) and Yes, FGF-R1 and the EPH-A2 receptor (Results not shown). Since a concentration of 40 μ M

in the culture medium is needed to inhibit AMPK completely in cells (Grahame Hardie, personal communication), the use of this compound to identify potential functions of AMPK is not recommended.

Inhibitors of IKK β (PS1145, BMS 345541 and SC 514).

These compounds have been described and used as inhibitors of the I κ B α kinases (IKKs) in many studies. PS 1145 inhibited IKK β with an IC₅₀ value of 0.25 μ M (Table 5). It also inhibited PIM1 and PIM3 with similar potency to IKK β and several other protein kinases with lower potency, but did not inhibit the other three members of the IKK subfamily, IKK α , IKK ϵ or TBK1 significantly (Table 7). BMS 345541 and SC514, inhibited IKK β about 10-fold more weakly than PS 1145 and also did not inhibit IKK α , IKK ϵ and TBK1 (Tables 5 and 7). BMS345541 inhibited several other kinases with slightly lower potency than IKK β , including ERK8, PKD1, CDK2 and CK1, while SC514 inhibited PIM3, PIM1, DYRK1A, DYRK3 and Aurora B similarly to IKK β (Table 7).

When added to the cell culture medium at 50 μ M, PS1145 was reported to suppress the LPS-induced the phosphorylation and activation of the protein kinase COT/Tp12 at Thr290 [85] leading to the conclusion that the phosphorylation of this residue was catalysed by IKK β . However, at a lower concentration (15 μ M) no suppression of IL-1-induced phosphorylation of Thr290 was observed, even though IKK β was still blocked completely, as shown by suppression of the degradation of I κ B α . This suggested that Thr290 is phosphorylated by a protein kinase distinct from IKK β [86], the blockade of Thr290 phosphorylation observed at higher (50 μ M) PS1145 presumably resulting from the “non-specific” inhibition of another protein kinase.

These results indicate that results obtained by using PS 1145 should be interpreted with caution and that the development of more specific inhibitors of IKK isoforms would be extremely useful.

The JNK inhibitors SP600125 and AS 601245.

We have reported previously that SP 600125 is not a specific inhibitor of JNK since it inhibited 13 of the 30 protein kinases tested with similar or greater potency than JNK isoforms [2]. However, despite the availability of this information, many laboratories have continued to use SP 600125 as a JNK inhibitor. Further analysis against our extended panel confirmed the lack of specificity of this compound and identified a number of other protein kinases that are inhibited by SP 600125. Those inhibited as potently or more potently than JNK isoforms, include PKD1, CHK2, Aurora B and C, MELK, CK1, DYRK2, DYRK3 and HIPK3 (Table 7).

AS 601245 has also been reported as a JNK inhibitor displaying 10-20 fold selectivity over Src, c-Raf, CDK2-cyclin A and p38 α MAPK with little inhibition of 20 other protein kinases tested. The compound was also reported to inhibit the LPS-induced production of TNF α in mice, to show efficacy in a model of collagen-induced rheumatoid arthritis [87], and to promote cell survival after cerebral ischaemia [87]. However, when profiled against our panel AS 601245 was not selective for JNK and inhibited many protein kinases, including p38 δ MAPK, ERK8, SGK1, GSK3 β , CK2, DYRK1a and PIM isoforms. More detailed kinetic analysis revealed that AS601245 was an exceptionally potent inhibitor of PIM1, PIM3 and GSK3 with IC₅₀ values in the nanomolar range, that were 50-100-fold lower than the IC₅₀ values for JNK1 and JNK2 (Tables 5 and 7).

We recommend that the use of SP 600125 and AS 601245 as JNK inhibitors in cell based assays be discontinued. The development of a potent and specific inhibitor that can suppress the activities JNK isoforms in cells would be very useful.

The MNK inhibitor CGP 57380.

CGP 57380 has been described as an MNK inhibitor [88] and used in cell-based assays for this purpose in several studies. We found that this compound was a relatively weak inhibitor of MNKs with IC_{50} values in the low micromolar range. Against our extended panel, several protein kinases were inhibited with similar potency including MKK1, CK1 and BRSK2 (Tables 5 and 7). These studies indicate that CGP 57380 is not a specific inhibitor of MNK isoforms and results obtained from its use in cell-based assays are difficult to interpret.

The specificities of some bis-indoyl maleimides (LY 333531, UCN01, Ro 318220, Go 6976, KT 5720).

We have previously examined the specificities of a number of bis-indoyl maleimides against a smaller panel of protein kinases and found them to inhibit many protein kinases of the AGC subfamily, such as S6K1, RSK2, MSK1 and $PKC\alpha$ [1]. However, at least two of these compounds, UCN01 and LY333531 have entered clinical trials for the treatment of cancer and diabetic retinopathy, respectively, and indeed clinical trials of LY 333531 were only discontinued during Phase III. We therefore studied a few of these compounds against our extended panel (Table 8). These studies revealed that LY333531 was an extremely potent inhibitor of PIM1/PIM3 and RSK1/RSK2, as well as $PKC\alpha$, and that several other protein kinases were also strongly inhibited, such as PDK1 (Table S2). UCN01 was an extremely powerful inhibitor of

RSK1/RSK2, PRK2, CaMKK β , PHK, AMPK, MARK3, CHK1, PIM3, MST2 and PDK1, as well as PKC α , while both Ro 318220 and Go6976 were potent inhibitors of RSK1, RSK2, PRK2, PKC α , PKD1, MSK1, GSK3 β , CDK2-cyclin A and PIM3, as well as PKC α . Go 6976 potently inhibited many protein kinases, such as RSK1, CaMKK β , PHK, CHK1, Aurora B, MST2, and PAKs 4,5 and 6 (Table S2).

KT5720, which was originally described as an inhibitor of PKA, also inhibited many protein kinases. MKK1, PDK1, PHK, Aurora B and PIM3 were among the protein kinases inhibited most strongly by this compound (Table S2).

In summary, none of the bis-indoyl maleimides that we have tested are sufficiently specific to be useful as protein kinase inhibitors in cell-based assays.

The protein kinase inhibitor Rottlerin.

Rottlerin is a compound extracted from the Monkey-face tree, which grows in the tropical regions of India and has been used for a variety of medicinal purposes over the generations. Although this compound was originally reported to inhibit PKC isoforms, especially PKC δ [89], and has been used as such in many publications, we failed to observe any inhibition of PKC α or PKC δ in a previous study and instead found that MAPKAP-K2 and PRAK were inhibited by this compound [1]. When Rottlerin was examined against our extended panel, many more protein kinases were found to be inhibited (Table S2), those suppressed most strongly being CHK2, PLK1, PIM3 and SRPK1. These observations indicate that rottlerin is too weak and non-specific an inhibitor to be useful in cell-based studies.

Inhibitors of ROCK (H7, H8, HA1077, H89, H1152, Y27632).

Isoquinoline sulphonamide derivatives, such as H7 and H8, developed by Hiroyoshi Hidaka and his colleagues were among the first inhibitors of protein kinases to be described [90] and the specificities of six of these compounds is shown in Table S2. Of these, H89 is marketed as a relatively selective inhibitor of PKA, while HA 1077 has been reported to inhibit the Rho-dependent protein kinases PRK1 [91] and ROCK [92], and Y27632 to inhibit ROCK1 and ROCK2 [93]. HA 1077 (also called Fasudil) has been approved in Japan for the treatment of cerebral vasospasm [94], while Y27632 has been reported to normalise blood pressure in rodent models of hypertension [93], perhaps by preventing ROCK from inhibiting the major myosin phosphatase in smooth muscle [95]. Y27632 also inhibits RhoA-mediated cell transformation [96], tumour cell invasion [97] and neutrophil chemotaxis [92], suggesting that inhibitors of ROCK may have therapeutic value as anti-cancer and anti-inflammatory agents.

We have previously examined the specificities of H89, HA1077 and Y27632 against a panel of 24 protein kinases [1] and here extend the analysis to 70 kinases. H7, H8, H89, HA1077 and H1152 not only inhibited ROCK2 and PRK2, but also other members of the AGC subfamily of protein kinases, such as RSK1, RSK2, PKA and MSK1 with similar or slightly lower potency than ROCK2 and PRK2. The compounds H7, H89 and HA1077 also inhibited AMPK and PKD1, while H89 also inhibited PKB isoforms and S6K1, and H-1152 inhibited PHK, Aurora B and Aurora C (Table S2). H89 has also been reported to inhibit voltage dependent potassium ion currents directly by blocking the pore cavity, an effect that was unrelated to the inhibition of PKA [98].

In summary, results obtained by the use of isoquinoline sulphonamides should be interpreted with caution.

Concluding remarks.

In this study, we have examined the specificities of many protein kinase inhibitors against a panel of 70-80 protein kinases. The results obtained have re-emphasised the need for great caution in using small molecule inhibitors of protein kinases to assess the physiological roles of these enzymes. Despite being used widely, many of the compounds analysed in this study were found to be too non-specific for useful conclusions to be made, other than to exclude the involvement of particular protein kinases in cellular processes. However, extrapolating data obtained from assays performed *in vitro* to make recommendations about the usefulness of these compounds as inhibitors of particular protein kinases in cells is not straightforward and depends on many factors, such as the stability and cell permeability of the compound, whether it accumulates in the plasma membrane or an intracellular organelle where a particular target is located, the concentration of the protein kinase *in vivo* and whether the compound is ATP competitive. The great majority of protein kinase inhibitors that have been developed bind at or near the ATP binding site (PD 184352, PD0325901, Akt-I-1,2 and rapamycin are exceptions) and, if they were purely ATP competitive, might be expected to be far less potent in cells where ATP concentrations are in the millimolar range, 100-fold higher than those for assays *in vitro*. However, this is not always the case, because the specificities of compounds frequently stem from their ability to bind not just in the ATP-binding pocket, but also in neighbouring hydrophobic pockets. Such interactions can induce fast or slow conformational changes and can lock the protein

kinase into an inactive state [29, 99]. Moreover, some compounds may bind more strongly to the inactive than the active conformation and, like the MKK1 inhibitor PD 98059 [45], prevent the protein kinase from being activated. In these situations the concentration of a compound needed to suppress activity in cells may be similar to or even lower than those that inhibit the protein kinase in vitro. Furthermore, a few compounds (such as Akt-I-1,2) only inhibit the full length protein kinase and not the catalytic domain (the reverse is also theoretically possible). Although catalytic domains are frequently employed for screening purposes if the full length protein is difficult to express, it should be born in mind that the use of truncated forms of proteins for screening or to assess specificity may sometimes give misleading results. A further caveat in extrapolating the data obtained in vitro to the cellular context is that only 70-80 protein kinases were used in the present study. Although this is a substantial number, it represents less than 20% of the protein kinases encoded by the human genome.

Therefore some compounds may be less specific than the results presented in Tables 1-8 would indicate. For this reason, the effects of two structurally unrelated inhibitors of the same protein kinase should be used on cells, wherever possible. Off-target effects can also be controlled for by the use of cells that do not express a particular protein kinase or that express a drug-resistant mutant of the protein kinase [27].

Despite the reservations outlined above, a number of compounds were identified that displayed impressive selectivity for a particular protein kinase. Based on the studies reported here and the effects of these compounds on cells that we and our colleagues have studied, we recommend that the following compounds be used in cells to assess the roles of particular protein kinases:- for p38 α /p38 β MAPKs use SB 203580 (1-5 μ M) and

BIRB 0796 (0.1 μM) in parallel; for substrates of p38 γ MAPK, identify proteins whose phosphorylation is prevented by BIRB 0796 (1.0 μM) but not by SB 203580 (5 μM); for PI 3-kinase use PI-103 (0.5 μM) and wortmannin (0.1 μM) in parallel; for PKB/AKT use Akt-I-1, 2 (1 μM); for MKK1, use PD 184352 (2 μM) or PD 0325901 (0.1 μM), for TORC1 use rapamycin (100 nM); for GSK3 use CT 99021 (2 μM), for RSK use BI-D1870 (10 μM) plus either SL0101 [54] or FMK [53, 55]; for CK1 use D4476 (50-100 μM). VX 680 (1 μM) may be used to inhibit Aurora kinases in cells [64] while roscovitine and purvalanol should continue to be useful as pan CDK inhibitors. Harmine is a very potent and selective inhibitor of DYRK1A, but whether it can be used to assess the role of this protein kinase in cells has yet to be assessed. It should be noted that some of the recommended inhibitors are not yet available commercially and must be synthesised.

It has been estimated that about 30% of the research and development programmes of the pharmaceutical industry are currently focussed on protein kinases (even higher in the field of cancer) and a huge number of compounds with impressive selectivity for particular protein kinases have been developed. The exploitation of these compounds for basic fundamental research by academic scientists is undoubtedly going to be of immense value in the future in facilitating our understanding of the roles of protein kinases in signal transduction pathways.

Future updates about the specificities of protein kinase inhibitors will appear on the MRC Protein Phosphorylation Unit website (<http://www.dundee.ac.uk/lifesciences/mrcppu>)

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Figure Legends

Figure 1. Effect of MKK1/MKK5 inhibitors on the activation of ERK1/ERK2 and ERK5. HeLa cells were serum starved for 16 h and incubated for a further 1 h in the presence (+) or absence (-) of PD184352, PD 0325901-C1 (R stereoisomer), PD 0325901 (S stereoisomer) or PD 0325901 at the concentrations indicated. The cells were then stimulated for 10 min with 100 ng/ml EGF, lysed and 30 µg of extract protein, denatured in SDS, subjected to SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted phosphorylated ERK1 and ERK2 (pERK1, pERK2) in A and immunoblotted with an antibody that recognises all forms of ERK5 equally well in B.

Figure 2. The effect of PI 103 on the activities of PI 3-kinase superfamily members in HEK293 cells. (A) HEK293 cells were incubated for 1 h with (+) or without (-) 0.5 µM PI103, then stimulated for 30 min with IGF-1 (50 ng/ml) and lysed. Cell extracts (60 µg protein) were denatured in SDS, subjected to SDS-PAGE and, after transfer to PVDF membranes, immunoblotted with a phospho-specific antibody that recognises PKB phosphorylated at Ser473. (B) HEK293 cells were incubated for 1 h with (+) or without (-) 0.5 µM PI 103, then treated for 2h with methylmethane sulphonate (MMS, 2mM) and lysed. Cell extracts (60 µg protein) were denatured in SDS, subjected to SDS-PAGE and, after transfer to PVDF membranes, immunoblotted with phospho-specific antibodies that recognise Checkpoint kinase 1 (CHK1) phosphorylated at Ser345 and CHK2 phosphorylated at Thr68.

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Table 1 The specificities of compounds developed as inhibitors of p38 α MAPK, Raf and Src family kinases

The concentrations of compounds used in the assays are indicated below each molecule and the results shown are presented as % activity remaining in the presence of inhibitor as compared to control incubations with inhibitor omitted (averages of duplicate determinations). Each experiment was repeated two or three times with similar results. Further details of the assays are given under experimental procedures. Important values are highlighted in boldface type. Abbreviation: Src-11, Src inhibitor 1; ND, not determined.

Kinase	SB203580 1 μ M	SB202190 1 μ M	BIRB0796 1 μ M	SU6656 1 μ M	Src-11 1 μ M	PP1 1 μ M	PP2 1 μ M	NA-PP1 1 μ M	NA-PP1 10 μ M	NM-PP1 1 μ M	NM-PP1 10 μ M	ZM336372 10 μ M	BAY439006 10 μ M	GW5074 1 μ M
MKK1	85 \pm 8	70 \pm 9	55 \pm 4	78 \pm 2	50 \pm 8	67 \pm 5	76 \pm 2	86 \pm 5	65 \pm 4	113 \pm 2	76 \pm 7	106 \pm 1	74 \pm 3	73 \pm 7
ERK1	89 \pm 0	104 \pm 0	97 \pm 0	115 \pm 1	103 \pm 8	113 \pm 1	104 \pm 1	104 \pm 1	70 \pm 1	85 \pm 6	46 \pm 2	81 \pm 1	92 \pm 7	99 \pm 3
ERK2	83 \pm 7	90 \pm 8	95 \pm 10	95 \pm 1	75 \pm 9	94 \pm 2	85 \pm 1	82 \pm 5	62 \pm 1	65 \pm 4	32 \pm 8	93 \pm 3	91 \pm 3	89 \pm 6
JNK1	95 \pm 0	83 \pm 2	90 \pm 2	94 \pm 5	84 \pm 3	93 \pm 10	92 \pm 4	92 \pm 2	93 \pm 2	90 \pm 0	85 \pm 4	85 \pm 2	83 \pm 5	94 \pm 5
JNK2	60 \pm 3	42 \pm 1	4 \pm 0	87 \pm 1	94 \pm 9	85 \pm 4	81 \pm 5	85 \pm 1	57 \pm 4	79 \pm 1	64 \pm 5	85 \pm 6	65 \pm 4	96 \pm 8
JNK3	72 \pm 7	49 \pm 0	69 \pm 0	95 \pm 0	81 \pm 7	85 \pm 0	66 \pm 0	93 \pm 3	88 \pm 3	98 \pm 9	80 \pm 1	86 \pm 0	101 \pm 4	91 \pm 2
p38 α MAPK	9 \pm 0	3 \pm 0	4 \pm 0	85 \pm 2	83 \pm 4	43 \pm 2	49 \pm 4	49 \pm 2	17 \pm 5	41 \pm 2	8 \pm 2	8 \pm 1	14 \pm 2	77 \pm 2
P38 β MAPK	13 \pm 1	5 \pm 3	13 \pm 4	87 \pm 5	90 \pm 8	38 \pm 2	48 \pm 0	77 \pm 2	24 \pm 2	53 \pm 5	15 \pm 5	15 \pm 2	22 \pm 4	100 \pm 4
p38 γ MAPK	104 \pm 1	86 \pm 7	36 \pm 4	102 \pm 3	104 \pm 2	98 \pm 8	107 \pm 2	97 \pm 1	81 \pm 2	96 \pm 1	99 \pm 2	95 \pm 1	68 \pm 1	107 \pm 7
p38 δ MAPK	107 \pm 3	87 \pm 1	40 \pm 5	86 \pm 1	86 \pm 2	69 \pm 7	93 \pm 2	108 \pm 1	80 \pm 2	98 \pm 1	94 \pm 4	95 \pm 9	61 \pm 4	97 \pm 4
ERK8	93 \pm 9	106 \pm 3	95 \pm 6	66 \pm 1	82 \pm 7	94 \pm 2	107 \pm 3	81 \pm 1	59 \pm 5	77 \pm 0	49 \pm 0	88 \pm 1	7 \pm 2	68 \pm 2
RSK1	52 \pm 8	78 \pm 2	35 \pm 2	56 \pm 1	73 \pm 5	94 \pm 4	118 \pm 0	89 \pm 3	53 \pm 5	84 \pm 2	56 \pm 7	70 \pm 1	41 \pm 4	65 \pm 2
RSK2	93 \pm 6	89 \pm 1	49 \pm 7	50 \pm 5	80 \pm 2	99 \pm 1	86 \pm 6	96 \pm 2	71 \pm 1	98 \pm 1	68 \pm 2	69 \pm 4	54 \pm 3	70 \pm 2
PKK1	81 \pm 2	93 \pm 0	86 \pm 6	97 \pm 9	88 \pm 3	84 \pm 11	88 \pm 6	98 \pm 5	98 \pm 3	97 \pm 1	102 \pm 0	84 \pm 3	82 \pm 2	98 \pm 4
PKB α	89 \pm 11	89 \pm 3	90 \pm 8	95 \pm 5	80 \pm 12	95 \pm 0	98 \pm 0	102 \pm 9	50 \pm 1	83 \pm 2	63 \pm 2	105 \pm 1	94 \pm 1	90 \pm 3
PKB β	90 \pm 1	91 \pm 6	89 \pm 2	86 \pm 4	86 \pm 7	97 \pm 11	90 \pm 1	96 \pm 2	89 \pm 1	98 \pm 5	92 \pm 4	63 \pm 1	83 \pm 1	82 \pm 5
SGK1	91 \pm 0	76 \pm 4	72 \pm 2	98 \pm 8	106 \pm 4	87 \pm 0	88 \pm 6	102 \pm 5	92 \pm 2	99 \pm 2	100 \pm 5	91 \pm 2	90 \pm 3	82 \pm 1
S6K1	80 \pm 5	83 \pm 1	99 \pm 6	83 \pm 1	92 \pm 4	42 \pm 5	61 \pm 5	86 \pm 7	61 \pm 7	91 \pm 7	82 \pm 7	111 \pm 3	76 \pm 4	98 \pm 6
PKA	89 \pm 4	73 \pm 1	69 \pm 4	98 \pm 6	95 \pm 9	52 \pm 3	50 \pm 5	86 \pm 3	56 \pm 2	32 \pm 2	7 \pm 1	93 \pm 6	72 \pm 3	109 \pm 2
ROCK 2	73 \pm 13	84 \pm 4	79 \pm 2	64 \pm 0	84 \pm 10	81 \pm 0	90 \pm 0	86 \pm 2	30 \pm 1	84 \pm 1	77 \pm 1	96 \pm 3	71 \pm 8	66 \pm 1
PRK2	95 \pm 8	65 \pm 4	74 \pm 7	37 \pm 7	81 \pm 4	84 \pm 3	104 \pm 7	110 \pm 1	90 \pm 2	109 \pm 5	98 \pm 2	92 \pm 6	79 \pm 1	91 \pm 6
PKC α	86 \pm 6	69 \pm 1	62 \pm 3	92 \pm 1	95 \pm 2	75 \pm 4	71 \pm 3	78 \pm 4	40 \pm 9	82 \pm 1	80 \pm 2	89 \pm 5	87 \pm 1	92 \pm 7
PKC ζ	89 \pm 0	113 \pm 5	116 \pm 6	97 \pm 3	95 \pm 4	93 \pm 7	86 \pm 6	86 \pm 0	84 \pm 1	83 \pm 2	85 \pm 5	78 \pm 8	92 \pm 4	103 \pm 9
PKD1	78 \pm 7	61 \pm 4	93 \pm 7	90 \pm 1	81 \pm 4	76 \pm 2	75 \pm 8	35 \pm 2	8 \pm 3	13 \pm 0	3 \pm 1	104 \pm 3	90 \pm 1	99 \pm 4
MSK1	91 \pm 10	87 \pm 0	84 \pm 5	88 \pm 1	81 \pm 3	72 \pm 12	83 \pm 1	81 \pm 6	47 \pm 6	78 \pm 1	70 \pm 3	96 \pm 1	84 \pm 3	80 \pm 4
MNK1	109 \pm 10	87 \pm 5	79 \pm 2	88 \pm 2	81 \pm 1	76 \pm 1	78 \pm 7	110 \pm 1	96 \pm 1	110 \pm 9	106 \pm 0	86 \pm 1	67 \pm 5	90 \pm 6
MNK2	107 \pm 1	103 \pm 6	91 \pm 0	102 \pm 2	61 \pm 7	97 \pm 1	107 \pm 1	106 \pm 1	85 \pm 8	115 \pm 1	98 \pm 5	102 \pm 8	40 \pm 3	91 \pm 5
MAPKAP-K2	84 \pm 2	122 \pm 4	105 \pm 3	107 \pm 7	81 \pm 3	101 \pm 1	72 \pm 1	117 \pm 0	98 \pm 4	99 \pm 3	115 \pm 7	89 \pm 6	104 \pm 5	84 \pm 7
MAPKAP-K3	120 \pm 5	104 \pm 2	89 \pm 3	105 \pm 9	88 \pm 4	98 \pm 3	101 \pm 1	91 \pm 1	88 \pm 5	96 \pm 1	94 \pm 2	105 \pm 8	92 \pm 2	95 \pm 5
FRAK	96 \pm 5	84 \pm 0	94 \pm 5	68 \pm 6	94 \pm 5	101 \pm 0	92 \pm 8	89 \pm 8	76 \pm 0	90 \pm 1	62 \pm 1	61 \pm 7	84 \pm 5	97 \pm 8
CAMKK α	106 \pm 14	94 \pm 1	96 \pm 4	20 \pm 4	ND	92 \pm 17	99 \pm 1	94 \pm 6	76 \pm 9	100 \pm 1	89 \pm 5	101 \pm 2	97 \pm 1	51 \pm 6
CAMKK β	99 \pm 5	86 \pm 1	88 \pm 2	13 \pm 6	94 \pm 1	96 \pm 3	89 \pm 1	90 \pm 2	72 \pm 8	103 \pm 7	76 \pm 2	96 \pm 2	88 \pm 3	63 \pm 3
CAMK1	86 \pm 6	91 \pm 1	84 \pm 6	99 \pm 3	86 \pm 9	90 \pm 3	91 \pm 0	83 \pm 3	63 \pm 3	83 \pm 6	47 \pm 1	77 \pm 9	53 \pm 3	89 \pm 2
SmMLCK	85 \pm 1	93 \pm 1	95 \pm 1	94 \pm 12	85 \pm 14	92 \pm 9	91 \pm 3	78 \pm 0	61 \pm 5	81 \pm 7	72 \pm 1	85 \pm 8	84 \pm 1	68 \pm 5
PHK	104 \pm 3	75 \pm 5	68 \pm 7	55 \pm 6	80 \pm 5	85 \pm 7	91 \pm 0	85 \pm 1	75 \pm 1	80 \pm 6	78 \pm 4	120 \pm 4	90 \pm 2	79 \pm 2
CHK1	66 \pm 6	85 \pm 5	73 \pm 3	62 \pm 7	86 \pm 8	90 \pm 6	93 \pm 2	97 \pm 8	96 \pm 2	96 \pm 8	106 \pm 0	89 \pm 8	89 \pm 5	85 \pm 8
CHK2	92 \pm 4	99 \pm 4	117 \pm 0	29 \pm 3	11 \pm 1	101 \pm 6	103 \pm 1	75 \pm 3	32 \pm 2	83 \pm 2	44 \pm 3	80 \pm 4	99 \pm 1	75 \pm 4
GSK3 β	34 \pm 3	27 \pm 2	54 \pm 8	88 \pm 6	89 \pm 1	94 \pm 6	107 \pm 1	95 \pm 1	77 \pm 0	90 \pm 3	93 \pm 3	107 \pm 4	64 \pm 1	54 \pm 3
CDK2-Cyclin A	104 \pm 3	82 \pm 7	83 \pm 6	69 \pm 5	97 \pm 13	89 \pm 6	87 \pm 1	95 \pm 3	56 \pm 1	86 \pm 5	43 \pm 5	101 \pm 4	80 \pm 7	97 \pm 3
PLK1	97 \pm 1	86 \pm 8	86 \pm 9	89 \pm 2	90 \pm 3	87 \pm 9	86 \pm 5	95 \pm 3	95 \pm 6	95 \pm 5	94 \pm 0	95 \pm 3	86 \pm 2	81 \pm 7
AURORA-B	85 \pm 2	90 \pm 9	82 \pm 2	10 \pm 6	35 \pm 2	73 \pm 1	93 \pm 6	91 \pm 8	75 \pm 8	106 \pm 3	83 \pm 0	95 \pm 3	15 \pm 1	60 \pm 2
AURORA-C	92 \pm 0	82 \pm 2	78 \pm 7	7 \pm 0	71 \pm 1	48 \pm 5	83 \pm 1	88 \pm 6	56 \pm 1	76 \pm 1	74 \pm 2	85 \pm 6	34 \pm 1	92 \pm 4
AMPK	80 \pm 0	91 \pm 3	75 \pm 3	10 \pm 6	87 \pm 11	102 \pm 9	113 \pm 1	82 \pm 6	73 \pm 2	86 \pm 3	97 \pm 2	82 \pm 2	89 \pm 4	97 \pm 4
MARK3	98 \pm 2	66 \pm 2	63 \pm 4	44 \pm 7	92 \pm 10	128 \pm 5	126 \pm 8	67 \pm 2	30 \pm 1	72 \pm 6	55 \pm 2	122 \pm 9	83 \pm 0	92 \pm 1
BRSK2	86 \pm 11	71 \pm 3	47 \pm 6	10 \pm 0	92 \pm 11	90 \pm 8	86 \pm 4	108 \pm 4	75 \pm 1	101 \pm 3	97 \pm 5	78 \pm 9	80 \pm 2	93 \pm 2
MELK	83 \pm 9	63 \pm 3	61 \pm 3	73 \pm 5	76 \pm 7	58 \pm 1	57 \pm 1	84 \pm 2	28 \pm 0	66 \pm 2	24 \pm 0	82 \pm 9	56 \pm 6	78 \pm 4
CK1 δ	8 \pm 1	21 \pm 1	97 \pm 8	104 \pm 6	101 \pm 4	20 \pm 2	7 \pm 1	30 \pm 0	4 \pm 1	56 \pm 5	14 \pm 3	90 \pm 4	99 \pm 4	93 \pm 5
CK2	98 \pm 2	103 \pm 3	96 \pm 5	109 \pm 1	92 \pm 2	99 \pm 4	108 \pm 4	88 \pm 0	87 \pm 0	80 \pm 7	92 \pm 3	93 \pm 3	95 \pm 3	68 \pm 3
DYRK1A	84 \pm 0	99 \pm 5	97 \pm 5	70 \pm 1	102 \pm 6	97 \pm 6	96 \pm 1	91 \pm 4	77 \pm 8	92 \pm 9	66 \pm 3	75 \pm 6	82 \pm 8	

Table 2. Potencies of compounds developed as Raf and Src inhibitors towards a variety of protein kinases. IC₅₀ values were determined from assays carried out at ten different inhibitor concentrations.

Compound	Protein Kinase	IC ₅₀ (μM)	ATP in assay (μM)
SU 6656	Src	0.10	50
SU 6656	Lck	0.15	50
SU 6656	Aurora B	0.019	20
SU 6656	Aurora C	0.017	5
SU 6656	BRSK2	0.10	50
SU 6656	MST2	0.11	50
SU 6656	AMPK	0.11	50
Src-I1	Src	0.18	50
Src-I1	RIP2	0.026	100
PP1	Src	0.053	50
PPI	Lck	0.040	50
PP1	RIP2	0.026	100
PP1	CK1δ	0.17	20
PP1	CSK	0.64	20
PP2	Src	0.036	50
PP2	Lck	0.031	50
PP2	RIP2	0.019	100
PP2	CK1δ	0.041	20
NA-PP1	JNK1[M108A]	0.27	20
NA-PP1	JNK1[M108G]	0.62	20
NA-PP1	Src	0.34	50
NA-PP1	Lck	0.66	50
NA-PP1	CSK	1.97	20
NA-PP1	RIP2	0.12	100
NA-PP1	PKD1	0.90	50
NA-PP1	CKI	0.15	20
NM-PP1	JNK1[M108A]	0.25	20
NM-PP1	JNK1[M108G]	0.14	20
NM-PP1	Src	0.62	50
NM-PP1	Lck	0.46	50
NM-PP1	CSK	0.51	20
NM-PP1	RIP2	0.13	100
NM-PP1	PKA	0.50	20
NM-PP1	PKD1	0.25	50
ZM 336372	c-RAF	0.031	100
ZM 336372	B-RAF	0.23	100
ZM 336372	Lck	0.57	50
ZM 336372	RIP2	>100	100
BAY 439006	c-RAF	0.37	100
BAY 439006	B-RAF	7.3	100
BAY 439006	p38αMAPK	3.2	100
BAY 439006	RIP2	3.6	100
GW 5074	c-RAF	0.17	100
GW 5074	B-RAF	0.19	100
GW 5074	PIM1	0.17	20

GW 5074	PIM2	0.07	5
GW 5074	PIM3	0.10	20

Stage 2(a) POST-PRINT

Table 3 The specificities of inhibitors of MKK1, Raf and GSK3

The concentrations of compounds used in the assays are indicated below each molecule and the results shown are presented as % activity remaining in the presence of inhibitors compared to control incubations with inhibitor omitted (averages of duplicate determinations). Each experiment was repeated two or three times with similar results. Further details of the assays are given under experimental procedures.

	U0126	PD184352	PD0325901	PD0325901-CI	BH-D1870	SL0101	FMK	CT 99021	ARAO14418	SB216763	SB415286	Alsterpaullone	Kanoeidone	ICI
	10 μM	10 μM	10 μM	10 μM	0.1 μM	10 μM	3 μM	1 μM	10 μM	2 μM	10 μM	1 μM	1 μM	10 mM
Kinase														
MKK1	24 ± 1	12 ± 1	5 ± 1	6 ± 5	98 ± 4	88 ± 7	80 ± 8	71 ± 3	77 ± 9	71 ± 6	28 ± 3	86 ± 3	88 ± 3	83 ± 8
ERK1	72 ± 5	86 ± 0	90 ± 1	104 ± 5	115 ± 2	98 ± 5	92 ± 6	77 ± 2	101 ± 1	99 ± 1	94 ± 9	95 ± 0	86 ± 7	101 ± 6
ERK2	68 ± 3	83 ± 6	91 ± 6	98 ± 7	85 ± 1	98 ± 9	79 ± 5	90 ± 6	95 ± 8	97 ± 7	96 ± 9	79 ± 5	88 ± 9	71 ± 7
JNK1	90 ± 2	92 ± 1	101 ± 12	90 ± 1	88 ± 6	95 ± 10	81 ± 3	85 ± 8	81 ± 2	106 ± 8	96 ± 1	82 ± 5	89 ± 9	81 ± 4
JNK2	73 ± 4	110 ± 4	96 ± 7	96 ± 9	115 ± 12	94 ± 7	83 ± 5	88 ± 3	72 ± 5	85 ± 6	88 ± 4	81 ± 4	83 ± 2	85 ± 8
JNK3	98 ± 4	108 ± 9	117 ± 1	82 ± 0	97 ± 10	98 ± 2	89 ± 4	71 ± 4	92 ± 0	88 ± 1	90 ± 6	78 ± 0	85 ± 0	77 ± 4
p38α MAPK	77 ± 6	86 ± 2	105 ± 7	93 ± 3	88 ± 13	94 ± 8	72 ± 2	82 ± 9	110 ± 9	77 ± 11	72 ± 6	109 ± 1	94 ± 5	100 ± 8
p38β MAPK	91 ± 9	96 ± 5	96 ± 5	107 ± 2	100 ± 4	98 ± 2	85 ± 4	87 ± 4	103 ± 8	85 ± 10	89 ± 7	86 ± 2	95 ± 1	102 ± 2
p38γ MAPK	90 ± 1	87 ± 2	103 ± 11	99 ± 1	111 ± 4	103 ± 8	92 ± 7	101 ± 7	89 ± 1	84 ± 9	98 ± 7	87 ± 5	86 ± 5	90 ± 1
p38δ MAPK	93 ± 7	93 ± 2	98 ± 14	80 ± 1	105 ± 8	88 ± 10	103 ± 9	100 ± 6	81 ± 3	81 ± 6	82 ± 2	86 ± 0	93 ± 7	78 ± 13
ERK8	67 ± 3	94 ± 2	67 ± 12	104 ± 7	82 ± 6	89 ± 9	58 ± 4	91 ± 1	37 ± 3	12 ± 1	13 ± 3	87 ± 5	75 ± 6	75 ± 5
RSK1	83 ± 6	124 ± 11	85 ± 11	99 ± 2	1 ± 1	23 ± 1	92 ± 6	65 ± 8	79 ± 4	50 ± 3	31 ± 2	67 ± 3	83 ± 3	80 ± 8
RSK2	81 ± 1	92 ± 4	81 ± 3	94 ± 3	2 ± 0	30 ± 5	74 ± 2	88 ± 6	84 ± 1	67 ± 7	29 ± 1	73 ± 7	83 ± 9	57 ± 8
PDK1	77 ± 3	79 ± 4	96 ± 1	84 ± 1	94 ± 8	103 ± 8	89 ± 10	86 ± 1	74 ± 1	92 ± 6	62 ± 2	71 ± 5	62 ± 3	94 ± 7
PKBα	76 ± 8	87 ± 7	94 ± 1	92 ± 1	98 ± 1	89 ± 8	82 ± 3	106 ± 1	95 ± 2	88 ± 9	92 ± 1	88 ± 1	95 ± 6	77 ± 5
PKBβ	98 ± 1	80 ± 0	116 ± 6	91 ± 5	102 ± 15	99 ± 4	97 ± 4	80 ± 6	86 ± 2	96 ± 9	85 ± 10	88 ± 1	90 ± 7	77 ± 6
SGK1	72 ± 6	101 ± 6	75 ± 8	91 ± 3	112 ± 7	110 ± 11	107 ± 8	79 ± 4	88 ± 4	100 ± 5	82 ± 6	85 ± 1	80 ± 0	119 ± 7
SGK1	93 ± 5	89 ± 1	106 ± 8	92 ± 1	119 ± 8	103 ± 8	33 ± 2	84 ± 2	75 ± 2	86 ± 11	81 ± 2	93 ± 1	114 ± 8	127 ± 14
PKA	93 ± 5	87 ± 0	108 ± 7	70 ± 3	89 ± 8	92 ± 7	85 ± 7	89 ± 0	68 ± 7	88 ± 6	80 ± 8	79 ± 1	63 ± 4	76 ± 0
ROCK 2	81 ± 6	86 ± 0	82 ± 4	100 ± 7	84 ± 14	109 ± 4	86 ± 7	79 ± 4	83 ± 2	101 ± 7	79 ± 4	87 ± 5	42 ± 6	109 ± 14
PRK2	85 ± 9	83 ± 5	101 ± 4	81 ± 7	97 ± 5	107 ± 7	83 ± 3	93 ± 1	85 ± 2	81 ± 7	43 ± 7	61 ± 6	58 ± 5	86 ± 8
PKCα	95 ± 5	88 ± 4	103 ± 5	89 ± 4	90 ± 9	90 ± 7	85 ± 7	78 ± 8	80 ± 3	73 ± 7	39 ± 3	80 ± 7	87 ± 6	90 ± 9
PKC ζ	78 ± 2	72 ± 3	97 ± 4	82 ± 5	80 ± 1	82 ± 3	91 ± 5	90 ± 2	70 ± 1	81 ± 3	69 ± 4	50 ± 4	72 ± 6	72 ± 2
PKD1	77 ± 6	85 ± 3	90 ± 7	83 ± 1	63 ± 11	89 ± 3	83 ± 4	75 ± 9	93 ± 5	100 ± 10	64 ± 8	91 ± 9	87 ± 1	95 ± 3
MEK1	85 ± 7	91 ± 5	87 ± 10	90 ± 1	125 ± 5	101 ± 1	75 ± 3	83 ± 9	81 ± 4	69 ± 2	62 ± 5	82 ± 6	88 ± 1	85 ± 6
MNK1	78 ± 2	92 ± 9	94 ± 2	92 ± 4	87 ± 9	82 ± 0	42 ± 2	81 ± 6	80 ± 3	85 ± 6	77 ± 8	74 ± 1	85 ± 3	53 ± 8
MNK2	85 ± 8	99 ± 3	104 ± 3	106 ± 3	96 ± 12	97 ± 6	65 ± 6	85 ± 9	95 ± 6	94 ± 3	91 ± 8	90 ± 4	102 ± 1	56 ± 3
MAPKAP-K2	95 ± 2	81 ± 5	112 ± 8	106 ± 8	89 ± 15	95 ± 4	94 ± 10	110 ± 4	103 ± 2	82 ± 2	85 ± 5	95 ± 4	92 ± 6	76 ± 7
MAPKAP-K3	97 ± 15	84 ± 8	114 ± 12	97 ± 1	108 ± 13	112 ± 12	92 ± 6	71 ± 8	82 ± 4	103 ± 2	99 ± 6	70 ± 5	97 ± 2	92 ± 12
PKAK	92 ± 2	82 ± 5	97 ± 7	81 ± 1	89 ± 15	82 ± 3	97 ± 5	76 ± 7	67 ± 5	90 ± 10	81 ± 5	76 ± 0	80 ± 1	75 ± 4
CAMKKα	99 ± 11	77 ± 1	80 ± 1	90 ± 1	92 ± 8	116 ± 9	70 ± 12	89 ± 3	79 ± 5	85 ± 4	83 ± 6	80 ± 3	74 ± 5	77 ± 4
CAMKKβ	92 ± 6	90 ± 1	97 ± 6	103 ± 1	73 ± 13	108 ± 7	49 ± 3	84 ± 2	73 ± 9	82 ± 4	29 ± 3	68 ± 3	61 ± 2	104 ± 8
CAMK1	69 ± 4	69 ± 4	109 ± 8	103 ± 1	103 ± 9	105 ± 8	77 ± 9	84 ± 1	76 ± 3	96 ± 10	61 ± 3	54 ± 2	88 ± 6	79 ± 4
Sm/MCLK	95 ± 5	105 ± 3	111 ± 2	82 ± 1	107 ± 4	109 ± 4	82 ± 4	83 ± 1	91 ± 3	60 ± 6	65 ± 8	91 ± 4	84 ± 1	43 ± 6
PHK	94 ± 7	98 ± 2	102 ± 11	83 ± 6	114 ± 14	97 ± 5	93 ± 5	75 ± 1	90 ± 4	87 ± 5	84 ± 3	11 ± 1	18 ± 1	60 ± 2
CHK1	83 ± 3	113 ± 1	97 ± 11	88 ± 9	73 ± 0	82 ± 1	88 ± 4	97 ± 2	83 ± 2	87 ± 6	60 ± 5	64 ± 4	72 ± 5	72 ± 5
CHK2	98 ± 3	95 ± 8	102 ± 5	87 ± 8	96 ± 3	99 ± 2	81 ± 9	93 ± 4	93 ± 3	93 ± 4	93 ± 4	13 ± 4	28 ± 2	59 ± 8
GSK3β	82 ± 5	78 ± 6	100 ± 15	76 ± 0	77 ± 5	87 ± 11	65 ± 6	1 ± 2	3 ± 0	2 ± 0	4 ± 1	3 ± 0	3 ± 1	30 ± 4
CDK2-Cyclin A	86 ± 1	88 ± 8	90 ± 5	94 ± 7	84 ± 0	106 ± 5	80 ± 7	53 ± 3	23 ± 3	52 ± 3	18 ± 1	10 ± 2	14 ± 0	100 ± 1
PLK1	92 ± 7	82 ± 0	105 ± 7	84 ± 8	5 ± 2	112 ± 11	82 ± 5	41 ± 2	76 ± 0	62 ± 6	85 ± 7	81 ± 5	90 ± 1	75 ± 4
AURORA B	86 ± 3	70 ± 1	87 ± 3	80 ± 3	22 ± 5	45 ± 7	59 ± 3	85 ± 9	71 ± 4	87 ± 3	64 ± 3	78 ± 3	49 ± 4	57 ± 2
AURORA C	88 ± 11	96 ± 9	118 ± 6	80 ± 3	76 ± 4	77 ± 2	75 ± 6	83 ± 7	84 ± 3	79 ± 7	70 ± 4	76 ± 4	83 ± 1	60 ± 2
AMPK	76 ± 12	108 ± 3	89 ± 6	114 ± 4	108 ± 6	88 ± 0	79 ± 4	85 ± 4	72 ± 1	67 ± 5	33 ± 4	73 ± 6	94 ± 1	103 ± 7
MARCK3	95 ± 5	98 ± 2	105 ± 5	101 ± 7	79 ± 5	113 ± 7	80 ± 3	91 ± 9	75 ± 5	76 ± 3	29 ± 2	63 ± 5	36 ± 5	89 ± 6
BRSK2	87 ± 12	82 ± 5	97 ± 7	87 ± 4	78 ± 9	80 ± 5	53 ± 6	67 ± 1	76 ± 4	83 ± 2	38 ± 3	37 ± 4	41 ± 0	81 ± 7
MELK	83 ± 11	82 ± 3	107 ± 1	73 ± 3	40 ± 5	102 ± 6	65 ± 8	45 ± 5	75 ± 4	78 ± 2	72 ± 5	54 ± 2	55 ± 6	82 ± 5
CK1α	90 ± 10	100 ± 1	103 ± 0	106 ± 4	81 ± 5	88 ± 1	63 ± 6	85 ± 4	89 ± 6	78 ± 5	68 ± 4	98 ± 4	98 ± 1	55 ± 10
CK2	99 ± 4	90 ± 8	100 ± 7	100 ± 7	97 ± 4	101 ± 7	92 ± 10	72 ± 2	79 ± 1	89 ± 3	97 ± 1	88 ± 1	78 ± 5	86 ± 4
DYRK1A	69 ± 4	80 ± 2	87 ± 11	84 ± 1	96 ± 9	80 ± 7	86 ± 11	81 ± 9	50 ± 6	8 ± 4	30 ± 3	83 ± 1	80 ± 4	89 ± 4
DYRK2	84 ± 3	76 ± 2	99 ± 1	110 ± 6	91 ± 1	89 ± 4	91 ± 8	87 ± 2	81 ± 9	92 ± 7	83 ± 7	87 ± 3	73 ± 5	94 ± 6
DYRK3	77 ± 4	104 ± 2	88 ± 8	79 ± 1	91 ± 3	85 ± 3	68 ± 7	66 ± 5	71 ± 1	79 ± 5	84 ± 5	93 ± 9	81 ± 4	88 ± 8
NEK2α	89 ± 7	98 ± 1	95 ± 10	99 ± 2	98 ± 10	101 ± 8	95 ± 4	83 ± 1	81 ± 3	74 ± 7	86 ± 6	92 ± 2	87 ± 1	74 ± 8
NEK6	90 ± 3	90 ± 6	106 ± 9	92 ± 4	115 ± 8	109 ± 13	90 ± 4	89 ± 5	91 ± 1	74 ± 4	74 ± 1	86 ± 1	93 ± 1	53 ± 12
NEK7	97 ± 6	93 ± 1	120 ± 6	99 ± 2	108 ± 0	105 ± 1	92 ± 8	85 ± 0	96 ± 3	85 ± 5	86 ± 9	92 ± 5	97 ± 7	73 ± 6
IKKβ	118 ± 7	92 ± 0	95 ± 5	89 ± 1	106 ± 11	100 ± 2	92 ± 8	88 ± 9	75 ± 3	99 ± 2	84 ± 5	90 ± 2	76 ± 4	88 ± 10
PIM1	87 ± 0	75 ± 3	86 ± 3	75 ± 1	76 ± 2	71 ± 3	61 ± 6	70 ± 2	83 ± 4	56 ± 10	37 ± 2	81 ± 5	49 ± 0	68 ± 1
PIM2	110 ± 3	92 ± 1	104 ± 5	90 ± 1	100 ± 8	95 ± 1	85 ± 10	63 ± 1	92 ± 5	81 ± 4	46 ± 3	91 ± 5	80 ± 1	120 ± 6
PIM3	63 ± 8	66 ± 4	62 ± 5	46 ± 2	65 ± 3	49 ± 2	56 ± 6	64 ± 8	69 ± 1	29 ± 7	46 ± 8	78 ± 8	28 ± 0	97 ± 2
SRPK1	102 ± 6	94 ± 4	95 ± 6	100 ± 5	94 ± 7	96 ± 5	85 ± 1	81 ± 8	74 ± 6	16 ± 1	54 ± 0	79 ± 7	92 ± 4	85 ± 12
MST2	96 ± 5	89 ± 1	99 ± 1	86 ± 5	41 ± 4	99 ± 2	76 ± 4	79 ± 7	80 ± 7	50 ± 7	14 ± 2	53 ± 3	41 ± 2	94 ± 5
EF2K	116 ± 5	97 ± 0	103 ± 4	99 ± 4	88 ± 0	86 ± 5	97 ± 8	99 ± 1	98 ± 5	98 ± 5	94 ± 2	87 ± 4	110 ± 1	58 ± 6
HIPK2	97 ± 3	151 ± 5	110 ± 2	98 ± 3	103 ± 7	72 ± 9	79 ± 11	84 ± 4	53 ± 3	15 ± 1	63 ± 2	77 ± 2	81 ± 9	66 ± 6
HIPK3	87 ± 7	95 ± 6	122 ± 2	102 ± 1	104 ± 5	110 ± 7	79 ± 9	89 ± 1	88 ± 1	41 ± 2	85 ± 6	95 ± 1	96 ± 0	50 ± 8
PAK4	94 ± 4	82 ± 3	99 ± 11	90 ± 1	81 ± 11	95 ± 5	91 ± 1	74 ± 1	66 ± 2	84 ± 13	52 ± 4	33 ± 0	8 ± 0	81 ± 6
PAK5	86 ± 2	98 ± 9	92 ± 7	75 ± 3	90 ± 5	86 ± 6	88 ± 6	80 ± 3	76 ± 2	85 ± 4	67 ± 2	40 ± 5	13 ± 2	81 ± 9
PAK6	85 ± 11	100 ± 1	129 ± 4	77 ± 7	102 ± 9	127 ± 9	93 ± 5	84 ± 1	74 ± 2	90 ± 5	74 ± 1	64 ± 9	39 ± 5	89 ± 2
Src	84 ± 6	90 ± 5	95 ± 1	97 ± 5	96 ± 3	105 ± 3	10 ± 1	83 ± 2	81 ± 1	83 ± 10	77 ± 7	61 ± 2	38 ± 1	62 ± 4
Lck	84 ± 4	85 ± 2	92 ± 10	88 ± 1	85 ± 0	88 ± 0	10 ± 1	88 ± 3	80 ± 1	77 ± 5	66 ± 3	52 ± 4	29 ± 3	76 ± 10
CSK	88 ± 12	81 ± 2	117 ± 3	82 ± 2	99 ± 9	105 ± 6	55 ± 7	66 ± 1	82 ± 2	88 ± 6	89 ± 2	83 ± 9	77 ± 5	89 ± 2
IKKα														51 ± 8
TBK1														54 ± 5

THIS IS NOT THE FINAL VERSION - see doi:10.1042/BJ20070797

Table 4 The specificities of inhibitors of PI 3-kinases, TORC1, PDK1 and PKB/AKT

The concentrations of compounds used in the assays are indicated below each molecule and the results are presented as % activity remaining in the presence of inhibitors compared to control incubations with inhibitor omitted (averages of duplicate determinations). Each experiment was repeated two or three times with similar results. Further details of the assays are given under experimental procedures. The effect of the inhibitor Akt-1,2 was tested on full length PKBa as well as the catalytic domain of PKBa for the reasons discussed in the text.

Kinase	Wortmannin		LY294002		PI-103		Rapamycin		BX795		BX795		BX320		BX320		A-443654		A-443654		Akt-1,2		Akt-1,2	
	1 μM	10 μM	1 μM	10 μM	1 μM	10 μM	1 μM	0.01 μM	0.1 μM	1 μM	10 μM	1 μM	10 μM	0.01 μM	0.1 μM	1 μM	10 μM	0.01 μM	0.1 μM	1 μM	10 μM	1 μM	10 μM	1 μM
MKK1	63 ± 0	86 ± 1	68 ± 5	93 ± 4	50 ± 4	33 ± 3	60 ± 7	30 ± 1	84 ± 2	67 ± 6	91 ± 8	76 ± 10												
EKK1	106 ± 5	85 ± 7	88 ± 6	100 ± 2	82 ± 9	66 ± 10	81 ± 9	72 ± 3	77 ± 8	59 ± 3	93 ± 6	100 ± 6												
EKK2	90 ± 7	88 ± 11	96 ± 4	94 ± 4	88 ± 4	71 ± 2	84 ± 1	73 ± 9	78 ± 6	64 ± 2	99 ± 12	76 ± 14												
JNK1	84 ± 2	92 ± 5	97 ± 3	90 ± 8	104 ± 7	85 ± 6	107 ± 8	79 ± 4	89 ± 7	90 ± 3	82 ± 12	70 ± 10												
JNK2	83 ± 0	97 ± 2	93 ± 4	81 ± 1	101 ± 12	70 ± 4	95 ± 4	65 ± 7	94 ± 1	78 ± 9	105 ± 3	81 ± 12												
JNK3	97 ± 0	86 ± 2	90 ± 6	81 ± 0	93 ± 4	93 ± 3	97 ± 6	102 ± 3	81 ± 9	86 ± 8	88 ± 5	80 ± 7												
p38α MAPK	117 ± 9	101 ± 10	91 ± 5	75 ± 4	86 ± 2	87 ± 1	107 ± 6	96 ± 7	131 ± 2	92 ± 6	101 ± 11	99 ± 14												
p38β MAPK	97 ± 5	100 ± 6	85 ± 5	84 ± 0	94 ± 13	71 ± 1	73 ± 7	46 ± 5	114 ± 3	105 ± 4	108 ± 10	114 ± 3												
p38γ MAPK	94 ± 1	89 ± 3	97 ± 8	81 ± 7	95 ± 2	77 ± 2	86 ± 4	80 ± 1	87 ± 5	83 ± 4	102 ± 12	93 ± 10												
p38δ MAPK	83 ± 5	102 ± 14	81 ± 4	92 ± 5	91 ± 6	76 ± 5	93 ± 2	75 ± 10	86 ± 1	68 ± 9	111 ± 11	98 ± 12												
ERK8	98 ± 6	70 ± 4	74 ± 3	97 ± 5	40 ± 3	7 ± 0	57 ± 10	19 ± 1	61 ± 2	28 ± 3	97 ± 12	71 ± 2												
RSK1	86 ± 5	92 ± 3	90 ± 13	90 ± 3	100 ± 6	55 ± 10	90 ± 3	40 ± 7	78 ± 1	32 ± 1	98 ± 5	67 ± 1												
RSK2	90 ± 2	104 ± 5	105 ± 10	97 ± 5	100 ± 9	75 ± 3	88 ± 3	42 ± 6	74 ± 3	27 ± 3	80 ± 1	74 ± 1												
PDK1	91 ± 1	89 ± 2	90 ± 2	85 ± 0	30 ± 2	5 ± 1	34 ± 1	8 ± 1	88 ± 1	79 ± 8	96 ± 3	95 ± 7												
PKBα	97 ± 6	95 ± 3	75 ± 2	91 ± 4	90 ± 14	90 ± 11	82 ± 15	57 ± 1	5 ± 2	3 ± 2	84 ± 10	64 ± 8												
PKBβ	90 ± 1	81 ± 9	77 ± 5	85 ± 9	113 ± 6	97 ± 7	104 ± 0	80 ± 1	17 ± 2	2 ± 0	92 ± 14	87 ± 15												
SGK1	86 ± 6	104 ± 4	89 ± 3	93 ± 1	87 ± 6	48 ± 3	75 ± 5	45 ± 2	78 ± 2	53 ± 5	116 ± 13	82 ± 3												
S6K1	95 ± 1	91 ± 3	82 ± 8	106 ± 8	100 ± 3	84 ± 9	100 ± 8	62 ± 6	88 ± 3	32 ± 6	100 ± 1	72 ± 6												
PKA	86 ± 1	97 ± 0	74 ± 6	77 ± 1	102 ± 1	72 ± 6	99 ± 1	59 ± 3	26 ± 1	3 ± 1	91 ± 6	77 ± 6												
ROCK 2	99 ± 3	90 ± 11	76 ± 4	103 ± 3	90 ± 6	28 ± 1	78 ± 4	56 ± 6	48 ± 3	8 ± 1	105 ± 11	92 ± 1												
PRK2	71 ± 2	75 ± 7	81 ± 9	79 ± 1	79 ± 5	43 ± 9	72 ± 2	42 ± 0	17 ± 2	2 ± 2	80 ± 15	68 ± 10												
PKCα	68 ± 1	77 ± 7	82 ± 5	88 ± 3	105 ± 1	74 ± 1	99 ± 2	68 ± 0	73 ± 3	29 ± 4	108 ± 4	87 ± 8												
PKCζ	84 ± 0	114 ± 5	77 ± 3	92 ± 6	96 ± 12	95 ± 2	83 ± 6	80 ± 8	82 ± 3	66 ± 0	105 ± 5	96 ± 9												
PKD1	92 ± 3	106 ± 3	92 ± 2	92 ± 3	84 ± 3	38 ± 3	90 ± 3	46 ± 11	63 ± 15	63 ± 3	23 ± 4	82 ± 15												
MSK1	85 ± 1	104 ± 0	97 ± 2	86 ± 2	88 ± 0	84 ± 5	92 ± 0	77 ± 8	19 ± 2	9 ± 2	81 ± 2	93 ± 14												
MNK1	81 ± 2	77 ± 5	90 ± 6	83 ± 2	80 ± 8	37 ± 3	88 ± 13	75 ± 4	120 ± 3	109 ± 2	77 ± 0	84 ± 2												
MNK2	97 ± 0	95 ± 4	104 ± 12	102 ± 3	47 ± 4	19 ± 2	72 ± 6	31 ± 2	97 ± 2	80 ± 5	99 ± 15	80 ± 3												
MAPKAP-K2	125 ± 4	103 ± 3	99 ± 17	108 ± 4	108 ± 8	108 ± 2	103 ± 8	68 ± 2	92 ± 8	73 ± 0	82 ± 7	60 ± 1												
MAPKAP-K3	98 ± 5	119 ± 5	88 ± 16	104 ± 5	103 ± 6	102 ± 1	106 ± 4	77 ± 6	91 ± 9	96 ± 2	86 ± 6	71 ± 5												
PRAK	69 ± 1	83 ± 6	93 ± 6	91 ± 3	97 ± 6	83 ± 2	83 ± 2	66 ± 10	107 ± 3	107 ± 4	85 ± 3	79 ± 6												
CAMKKα	83 ± 1	71 ± 4	88 ± 7	80 ± 8	86 ± 13	69 ± 6	89 ± 14	48 ± 4	112 ± 2	117 ± 1	93 ± 5	62 ± 3												
CAMKKβ	71 ± 4	71 ± 4	79 ± 6	83 ± 2	82 ± 6	30 ± 1	63 ± 10	20 ± 2	83 ± 8	63 ± 9	138 ± 10	108 ± 9												
CAMK1	97 ± 5	87 ± 15	108 ± 8	97 ± 1	86 ± 10	71 ± 3	71 ± 5	52 ± 6	89 ± 3	77 ± 6	43 ± 2	3 ± 0												
SmMLCK	72 ± 4	88 ± 7	62 ± 5	107 ± 1	110 ± 6	95 ± 1	104 ± 4	95 ± 4	69 ± 2	27 ± 2	79 ± 6	11 ± 4												
PHK	74 ± 2	94 ± 10	84 ± 6	95 ± 2	94 ± 12	62 ± 6	102 ± 2	77 ± 3	86 ± 7	54 ± 6	103 ± 13	101 ± 10												
CHK1	87 ± 7	83 ± 12	94 ± 6	97 ± 6	84 ± 5	46 ± 3	71 ± 5	34 ± 1	100 ± 3	99 ± 2	93 ± 11	85 ± 2												
CHK2	90 ± 3	84 ± 0	79 ± 12	92 ± 3	91 ± 1	46 ± 4	100 ± 2	45 ± 0	94 ± 8	95 ± 2	91 ± 12	83 ± 7												
GSK3β	65 ± 1	37 ± 3	84 ± 11	83 ± 6	82 ± 15	48 ± 1	55 ± 2	23 ± 0	81 ± 1	33 ± 4	89 ± 6	78 ± 10												
CDK2-Cyclin A	95 ± 9	81 ± 2	95 ± 5	92 ± 4	57 ± 7	10 ± 0	91 ± 7	35 ± 1	82 ± 5	31 ± 2	97 ± 0	97 ± 14												
PLK1	64 ± 1	26 ± 1	79 ± 10	78 ± 4	95 ± 7	92 ± 9	97 ± 8	70 ± 1	89 ± 5	80 ± 1	79 ± 3	90 ± 2												
AURORA-B	83 ± 1	70 ± 4	84 ± 2	86 ± 5	17 ± 1	3 ± 1	77 ± 3	4 ± 0	81 ± 3	80 ± 2	82 ± 9	60 ± 4												
AURORA-C	69 ± 3	84 ± 2	104 ± 4	97 ± 5	27 ± 3	3 ± 1	8 ± 1	7 ± 1	100 ± 4	92 ± 8	75 ± 6	66 ± 0												
AMPK	88 ± 3	97 ± 7	106 ± 16	87 ± 2	52 ± 1	11 ± 0	46 ± 4	13 ± 3	84 ± 8	67 ± 3	97 ± 10	92 ± 10												
MARK3	85 ± 3	79 ± 13	100 ± 6	88 ± 4	12 ± 1	3 ± 0	36 ± 4	8 ± 0	100 ± 3	83 ± 1	84 ± 12	95 ± 12												
BRSK2	80 ± 4	104 ± 4	97 ± 1	90 ± 4	92 ± 4	45 ± 3	72 ± 3	23 ± 4	88 ± 9	79 ± 1	96 ± 8	93 ± 13												
MELK	61 ± 2	86 ± 9	79 ± 9	74 ± 5	95 ± 4	32 ± 3	57 ± 10	11 ± 1	96 ± 2	63 ± 2	80 ± 2	68 ± 0												
CK1δ	94 ± 1	57 ± 3	87 ± 1	88 ± 1	79 ± 5	91 ± 1	89 ± 6	96 ± 6	95 ± 3	74 ± 3	105 ± 13	111 ± 3												
CK2	96 ± 7	23 ± 5	83 ± 6	92 ± 9	93 ± 11	93 ± 0	105 ± 10	95 ± 7	96 ± 0	90 ± 8	88 ± 13	91 ± 14												
DYRK1A	88 ± 1	76 ± 10	87 ± 5	91 ± 4	94 ± 7	83 ± 3	87 ± 4	64 ± 8	10 ± 2	7 ± 3	94 ± 15	94 ± 12												
DYRK2	98 ± 1	94 ± 3	81 ± 7	84 ± 9	105 ± 10	76 ± 6	93 ± 0	71 ± 0	79 ± 5	26 ± 1	113 ± 8	118 ± 1												
DYRK3	84 ± 1	78 ± 2	79 ± 8	88 ± 1	85 ± 9	65 ± 15	86 ± 9	44 ± 1	41 ± 3	8 ± 1	86 ± 4	75 ± 2												
NEK2a	81 ± 1	91 ± 8	114 ± 2	82 ± 5	92 ± 7	95 ± 1	95 ± 4	61 ± 4	101 ± 5	95 ± 2	91 ± 4	100 ± 4												
NEK6	113 ± 7	103 ± 5	111 ± 7	95 ± 3	96 ± 3	98 ± 3	117 ± 14	92 ± 2	95 ± 2	95 ± 4	88 ± 14	75 ± 4												
NEK7	100 ± 7	86 ± 1	106 ± 2	102 ± 5	111 ± 8	99 ± 2	116 ± 14	84 ± 11	86 ± 8	84 ± 1	91 ± 7	85 ± 10												
IKKβ	96 ± 4	68 ± 1	95 ± 5	82 ± 3	108 ± 2	93 ± 3	96 ± 10	69 ± 2	93 ± 8	89 ± 2	98 ± 15	90 ± 3												
PIM1	87 ± 3	8 ± 2	77 ± 3	91 ± 3	103 ± 7	103 ± 1	86 ± 2	64 ± 4	57 ± 8	15 ± 2	80 ± 1	57 ± 6												
PIM2	83 ± 4	65 ± 2	106 ± 7	117 ± 0	108 ± 3	99 ± 1	89 ± 3	72 ± 2	59 ± 0	15 ± 2	88 ± 3	89 ± 3												
PIM3	86 ± 1	13 ± 3	79 ± 2	88 ± 1	96 ± 7	63 ± 3	97 ± 2	54 ± 4	54 ± 6	13 ± 1	84 ± 10	60 ± 6												
SRPK1	73 ± 3	101 ± 5	82 ± 8	84 ± 1	99 ± 11	86 ± 15	79 ± 8	70 ± 2	92 ± 8	91 ± 2	84 ± 10	84 ± 10												
MST2	96 ± 9	85 ± 1	91 ± 6	90 ± 5	96 ± 2	92 ± 11	97 ± 4	70 ± 6	76 ± 4	29 ± 0	96 ± 11	78 ± 2												
EP2K	97 ± 4	83 ± 3	110 ± 13	94 ± 0	98 ± 5	93 ± 7	110 ± 7	84 ± 4	93 ± 7	94 ± 8	93 ± 4	40 ± 7												
HIPK2	94 ± 7	38 ± 2	67 ± 0	93 ± 7	95 ± 11	101 ± 8	97 ± 9	75 ± 3	73 ± 2	24 ± 3	102 ± 6	85 ± 3												
HIPK3	75 ± 1	77 ± 2	75 ± 6	108 ± 4	95 ± 1	94 ± 1	102 ± 7	101 ± 5	90 ± 0	71 ± 2	77 ± 2	66 ± 3												
PAK4	90 ± 2	67 ± 5	98 ± 5	81 ± 1	91 ± 15	71 ± 4	79 ± 7	26 ± 1	99 ± 3	90 ± 6	92 ± 4	49 ± 5												
PAK5	72 ± 1	92 ± 5	91 ± 8	89 ± 8	97 ± 3	81 ± 0	93 ± 1	41 ± 8	104 ± 2	96 ± 1	96 ± 1	61 ± 4												
PAK6	86 ± 1	100 ± 0	98 ± 7	89 ± 1	92 ± 3	85 ± 3	89 ± 2	61 ± 1	87 ± 2	87 ± 3	116 ± 7	92 ± 2												
Src	83 ± 1	106 ± 11	90 ± 8	86 ± 1	90 ± 4	77 ± 2	85 ± 1	40 ± 2	91 ± 0	86 ± 5	105 ± 10	118 ± 1												
Lck	73 ± 4	97 ± 2	89 ± 3	79 ± 7	97 ± 4	80 ± 11	76 ± 4	41 ± 2	97 ± 8	97 ± 3	84 ± 2	65 ± 6												
CSK	73 ± 9	104 ± 3	82 ± 1	83 ± 1	114 ± 12	101 ± 7	93 ± 15	67 ± 11	99 ± 2	87 ± 9	103 ± 15	103 ± 15												
IKKε																								
TBK1																								
PKBa-full length																								

THIS IS NOT THE FINAL VERSION - see doi:10.1042/BJ20070797

Table 5. Relative potencies of small molecule kinase inhibitors towards different protein kinases.
IC₅₀ values were determined from assays carried out at ten different inhibitor concentrations.

Compound	Protein Kinase	IC ₅₀ (μM)	ATP in assay (μM)
FMK	Src	0.56	50
FMK	Lck	0.75	50
FMK	S6K1	0.95	20
FMK	Yes	0.51	20
FMK	Eph-A2	0.76	50
Wortmannin	smMLCK	5.0	50
Wortmannin	PLK1	1.3	5
LY 294002	PLK1	2.0	5
LY 294002	PIM1	0.82	20
LY294002	PIM3	1.4	20
D4476	CK1	0.3	100
D4476	PKD1	9.1	50
D4476	p38αMAPK	5.8	50
Harmine	DYRK1A	0.08	50
Harmine	DYRK2	0.9	50
Harmine	DYRK3	0.8	50
Harmine	PIM3	4.3	20
Harmine	CK1	1.5	20
Roscovitine	CDK2	0.14	20
Roscovitine	PAK4	6.9	5
Purvalanol	CDK2	0.03	20
Purvalanol	PAK4	0.13	5
VX 680	Aurora B	0.04	100
VX 680	Aurora C	0.07	100
VX 680	Src	0.34	50
VX 680	MELK	0.42	50
SU6668	Aurora B	0.035	20
SU6668	Aurora C	0.21	5
STO 609	CaMKKβ	0.01	20
STO 609	CaMKKα	0.12	20
STO 609	MNK1	0.12	50
STO 609	CK2	0.19	5
STO 609	AMPK	0.16	50
STO 609	PIM2	0.11	5
STO 609	PIM3	0.083	100
STO 609	DYRK2	0.95	50
PS 1145	IKKβ	0.25	5
PS 1145	PIMI	1.1	20
PS 1145	PIM3	0.88	20
BMS 345541	IKKβ	2.6	5
SC514	IKKβ	2.0	5
AS 601245	JNK1	2.6	20
AS 601245	JNK2	5.0	20
AS 601245	GSK3	0.04	20
AS 601245	PIM1	0.08	20
AS 601245	PIM3	0.03	5
AS 601245	DYRK2	0.3	50
CGP 57380	MNK1	0.87	50
CGP 57380	MNK2	1.6	50
CGP 57380	CKI	0.51	20
CGP 57380	Aurora B	2.5	20
CGP 57380	DYRK3	3.2	5
CGP 57380	SGK1	2.7	20
CGP 57380	BRSK2	1.1	50
CGP 57380	Lck	2.5	50

Table 6 the specificities of compounds identified as CK1 and DYRK inhibitors

The concentrations of compounds used in the assays are indicated below each molecule and the results are presented as % activity remaining in the presence of inhibitor as compared to control incubations with inhibitor omitted (averages of duplicate determinations). Each experiment was repeated two or three times with similar results. Further details of the assays are given under experimental procedures.

Kinase	D4476 10 μ M	CK1-7 25 μ M	IC261 25 μ M	Harmine 1 μ M	Harmane 1 μ M	Harmalol 1 μ M	Harmaline 1 μ M
MKK1	93 \pm 2	76 \pm 2	86 \pm 2	73 \pm 4	90 \pm 6	86 \pm 1	95 \pm 3
ERK1	102 \pm 4	102 \pm 4	91 \pm 6	95 \pm 5	107 \pm 1	107 \pm 1	108 \pm 4
ERK2	85 \pm 2	100 \pm 2	93 \pm 4	95 \pm 5	95 \pm 6	95 \pm 6	87 \pm 0
JNK1	86 \pm 5	91 \pm 1	94 \pm 2	89 \pm 3	92 \pm 4	102 \pm 4	96 \pm 3
JNK2	77 \pm 7	87 \pm 9	101 \pm 3	91 \pm 5	84 \pm 6	99 \pm 6	86 \pm 7
JNK3	82 \pm 0	103 \pm 0	93 \pm 4	91 \pm 0	89 \pm 2	113 \pm 0	89 \pm 0
p38 α MAPK	38 \pm 1	103 \pm 5	92 \pm 4	100 \pm 7	74 \pm 1	96 \pm 2	88 \pm 4
P38 β MAPK	55 \pm 2	101 \pm 9	97 \pm 7	103 \pm 4	90 \pm 7	95 \pm 4	92 \pm 1
p38 γ MAPK	87 \pm 1	102 \pm 3	93 \pm 4	80 \pm 3	97 \pm 9	104 \pm 2	120 \pm 6
p38 δ MAPK	89 \pm 2	80 \pm 8	91 \pm 4	88 \pm 1	90 \pm 1	89 \pm 4	92 \pm 5
ERK8	80 \pm 4	29 \pm 6	68 \pm 2	80 \pm 1	70 \pm 0	62 \pm 3	44 \pm 4
RSK1	81 \pm 1	48 \pm 3	87 \pm 3	105 \pm 6	98 \pm 1	100 \pm 9	110 \pm 5
RSK2	82 \pm 1	63 \pm 4	89 \pm 1	104 \pm 3	94 \pm 1	83 \pm 8	112 \pm 1
PDK1	85 \pm 1	83 \pm 3	89 \pm 5	87 \pm 1	90 \pm 7	93 \pm 6	86 \pm 9
PKB α	107 \pm 9	54 \pm 1	94 \pm 5	101 \pm 7	104 \pm 3	89 \pm 2	95 \pm 0
PKB β	88 \pm 1	89 \pm 4	90 \pm 3	90 \pm 1	91 \pm 8	81 \pm 1	105 \pm 1
SGK1	73 \pm 2	25 \pm 2	79 \pm 4	83 \pm 1	86 \pm 4	86 \pm 3	85 \pm 7
S6K1	76 \pm 5	49 \pm 5	84 \pm 2	93 \pm 3	94 \pm 3	94 \pm 8	93 \pm 1
PKA	85 \pm 6	65 \pm 7	82 \pm 3	80 \pm 9	95 \pm 2	90 \pm 4	89 \pm 1
ROCK 2	105 \pm 1	65 \pm 6	78 \pm 0	80 \pm 4	104 \pm 1	91 \pm 8	79 \pm 6
PRK2	107 \pm 4	47 \pm 3	87 \pm 5	86 \pm 4	85 \pm 6	86 \pm 3	85 \pm 1
PKC α	94 \pm 5	86 \pm 3	90 \pm 2	82 \pm 4	90 \pm 5	92 \pm 1	90 \pm 9
PKC ζ	81 \pm 1	52 \pm 1	80 \pm 4	83 \pm 1	89 \pm 3	88 \pm 0	84 \pm 3
PKD1	43 \pm 4	60 \pm 3	62 \pm 3	80 \pm 9	88 \pm 1	111 \pm 3	108 \pm 1
MSK1	90 \pm 7	42 \pm 2	88 \pm 1	83 \pm 5	91 \pm #	96 \pm 1	94 \pm 1
MNK1	81 \pm 1	26 \pm 0	79 \pm 3	82 \pm 4	82 \pm 1	97 \pm 1	80 \pm 1
MNK2	88 \pm 4	76 \pm 0	97 \pm 5	80 \pm 4	112 \pm 4	106 \pm 5	92 \pm 2
MAPKAP-K2	99 \pm 4	85 \pm 3	88 \pm 4	110 \pm 4	109 \pm 3	88 \pm 1	89 \pm 4
MAPKAP-K3	98 \pm 3	86 \pm 7	94 \pm 3	98 \pm 6	104 \pm 6	99 \pm 9	97 \pm 5
PRAK	77 \pm 2	81 \pm 4	82 \pm 5	90 \pm 2	84 \pm 8	85 \pm 3	74 \pm 1
CAMKK α	89 \pm 1	86 \pm 4	67 \pm 4	95 \pm 4	86 \pm 6	103 \pm 1	92 \pm 1
CAMKK β	100 \pm 2	86 \pm 3	86 \pm 2	81 \pm 1	93 \pm 5	90 \pm 1	89 \pm 7
CAMK1	88 \pm 2	82 \pm 3	55 \pm 5	94 \pm 4	91 \pm 4	101 \pm 3	103 \pm 0
SmMLCK	81 \pm 5	84 \pm 7	70 \pm 2	87 \pm 7	100 \pm 4	107 \pm 9	112 \pm 2
PHK	89 \pm 4	72 \pm 5	86 \pm 1	85 \pm 5	102 \pm 3	87 \pm 3	84 \pm 7
CHK1	84 \pm 8	86 \pm 2	99 \pm 2	99 \pm 9	99 \pm 4	84 \pm 5	94 \pm 5
CHK2	92 \pm 5	64 \pm 1	76 \pm 4	100 \pm 0	92 \pm 3	122 \pm 1	117 \pm 5
GSK3 β	88 \pm 5	75 \pm 4	74 \pm 6	87 \pm 0	108 \pm 1	88 \pm 4	100 \pm 9
CDK2-Cyclin A	113 \pm 8	62 \pm 4	83 \pm 4	86 \pm 4	103 \pm 6	101 \pm 1	100 \pm 7
PLK1	90 \pm 9	81 \pm 4	95 \pm 1	97 \pm 1	81 \pm 2	83 \pm 1	80 \pm 1
AURORA B	88 \pm 300	78 \pm 0	75 \pm 3	81 \pm 2	106 \pm 3	90 \pm 8	90 \pm 4
AURORA C	81 \pm 4	95 \pm 3	94 \pm 3	83 \pm 1	87 \pm 3	91 \pm 6	99 \pm 8
AMPK	98 \pm 0	29 \pm 5	71 \pm 2	72 \pm 1	112 \pm #	110 \pm 1	106 \pm 1
MARK3	82 \pm 4	78 \pm 5	88 \pm 5	83 \pm 3	128 \pm 4	131 \pm 9	110 \pm 5
BRSK2	90 \pm 5	42 \pm 1	88 \pm 4	83 \pm 3	101 \pm 1	98 \pm 4	103 \pm 1
MELK	83 \pm 1	63 \pm 4	75 \pm 8	80 \pm 2	82 \pm 3	77 \pm 0	95 \pm 0
CK1 δ	4 \pm 0	12 \pm 1	9 \pm 2	60 \pm 4	99 \pm 6	94 \pm 9	65 \pm 5
CK2	83 \pm 5	65 \pm 1	97 \pm 2	68 \pm 5	85 \pm 8	91 \pm 1	87 \pm 1
DYRK1A	86 \pm 7	39 \pm 6	73 \pm 1	4 \pm 2	44 \pm 4	67 \pm 6	29 \pm 4
DYRK2	93 \pm 3	76 \pm 3	99 \pm 6	15 \pm 0	83 \pm 5	86 \pm 4	73 \pm 1
DYRK3	76 \pm 1	46 \pm 1	89 \pm 3	6 \pm 0	72 \pm 4	71 \pm 2	49 \pm 5
NEK2a	89 \pm 7	85 \pm 4	75 \pm 6	94 \pm 1	79 \pm 0	82 \pm 4	96 \pm 5
NEK6	79 \pm 1	90 \pm 2	95 \pm 5	87 \pm 1	90 \pm 1	93 \pm 1	94 \pm 1
NEK7	100 \pm 6	102 \pm 3	103 \pm 4	108 \pm 1	105 \pm 1	109 \pm 8	107 \pm 9
IKK β	63 \pm 3	87 \pm 2	90 \pm 5	73 \pm 9	76 \pm 8	74 \pm 1	94 \pm 5
PIM1	93 \pm 8	28 \pm 2	43 \pm 1	86 \pm 1	94 \pm 1	92 \pm 1	104 \pm 7
PIM2	90 \pm 4	55 \pm 6	75 \pm 1	88 \pm 7	85 \pm 1	101 \pm 1	117 \pm 1
PIM3	82 \pm 4	28 \pm 1	41 \pm 1	64 \pm 1	80 \pm 4	71 \pm 1	90 \pm 6
SRPK1	84 \pm 5	86 \pm 2	95 \pm 1	86 \pm 7	81 \pm 3	88 \pm 8	89 \pm 6
MST2	96 \pm 2	91 \pm 0	70 \pm 1	85 \pm 7	81 \pm 1	90 \pm 3	101 \pm 1
EF2K	100 \pm 1	94 \pm 6	100 \pm 4	104 \pm 8	101 \pm 3	102 \pm 6	107 \pm 2
HIPK2	80 \pm 4	98 \pm 7	96 \pm 6	108 \pm 7	104 \pm 5	101 \pm 1	91 \pm 1
HIPK3	96 \pm 3	86 \pm 7	95 \pm 3	87 \pm 8	104 \pm 0	93 \pm 8	97 \pm 1
PAK4	105 \pm 1	88 \pm 3	84 \pm 2	96 \pm 3	103 \pm 5	105 \pm 6	90 \pm 1
PAK5	100 \pm 1	86 \pm 7	84 \pm 1	92 \pm 5	97 \pm 8	95 \pm 4	84 \pm 7
PAK6	103 \pm 5	78 \pm 3	76 \pm 0	83 \pm 5	105 \pm 3	92 \pm 1	106 \pm 2
Src	82 \pm 0	92 \pm 1	70 \pm 4	88 \pm 9	83 \pm 2	85 \pm 5	94 \pm 6
Lck	82 \pm 5	83 \pm 6	49 \pm 4	81 \pm 2	93 \pm 1	93 \pm 1	81 \pm 1
CSK	81 \pm 6	79 \pm 5	81 \pm 4	86 \pm 3	81 \pm 5	84 \pm 7	82 \pm 7

Table 7 The specificities of compounds developed as inhibitors of CDK, Aurora kinases, CaMKK, AMPK, IKK, JNK and MNK

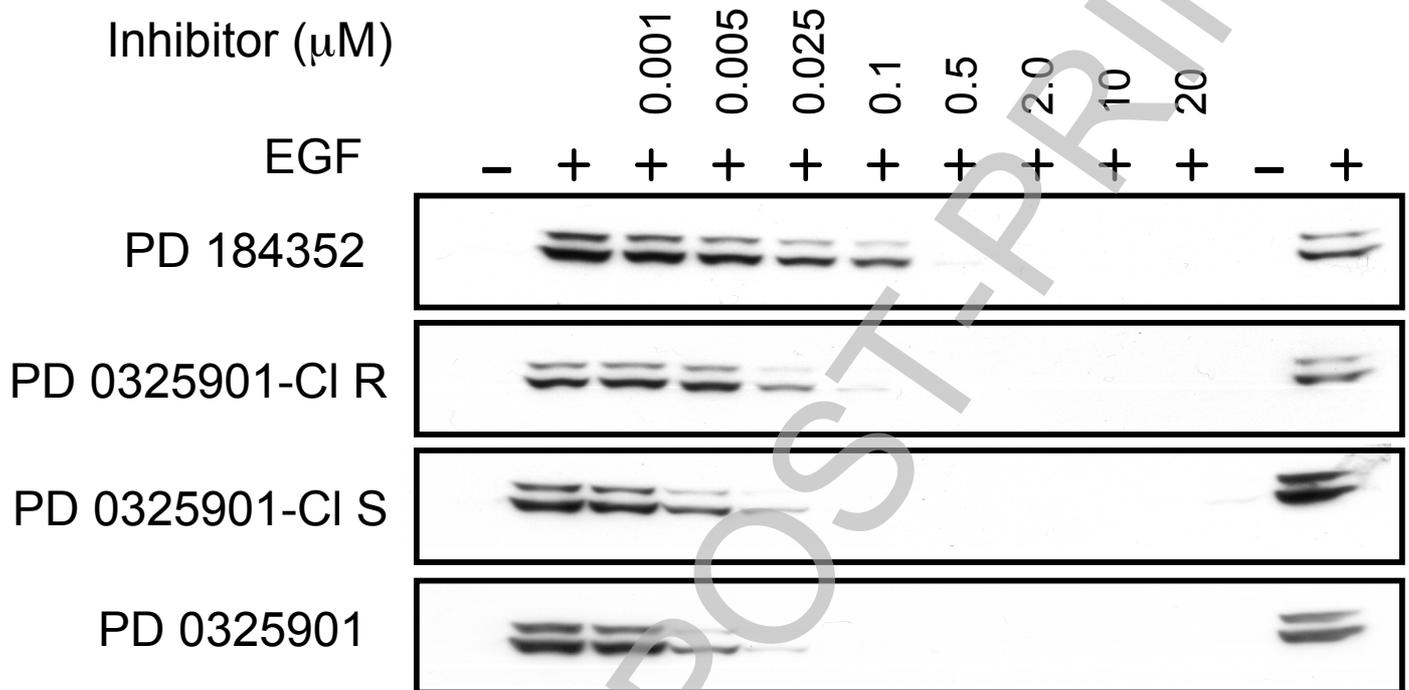
The concentrations of compounds used in the assays are indicated below each molecule and the results are presented as % activity remaining in the presence of inhibitors as compared to control incubations with inhibitor omitted (averages of duplicate determinations). Each experiment was repeated two or three times with similar results. Further details of the assays are given under experimental procedures.

	Roscovitine 1 μM	Purvalanol 0.1 μM	VX680 1 μM	SU6668 1 μM	STO609 1 μM	Compound C 1 μM	PS1145 10 μM	BMS-345541 10 μM	SCS14 10 μM	SP600125 1 μM	AS601245 1 μM	AS601245 10 μM	CGP57380 1 μM
Kinase	1 μM	0.1 μM	1 μM	1 μM	1 μM	1 μM	10 μM	10 μM	10 μM	1 μM	1 μM	10 μM	1 μM
MKK1	81 ± 6	104 ± 7	48 ± 1	31 ± 1	88 ± 5	97 ± 3	83 ± 1	66 ± 4	83 ± 8	58 ± 7	73 ± 6	51 ± 6	56 ± 1
ERK1	82 ± 2	95 ± 4	96 ± 5	85 ± 3	98 ± 9	110 ± 7	106 ± 8	87 ± 6	88 ± 6	89 ± 4	104 ± 5	97 ± 3	78 ± 4
ERK2	83 ± 2	79 ± 5	96 ± 1	92 ± 3	92 ± 3	117 ± 7	84 ± 3	92 ± 6	96 ± 6	86 ± 3	91 ± 9	68 ± 3	82 ± 3
JNK1	89 ± 5	100 ± 4	100 ± 2	99 ± 14	84 ± 4	97 ± 6	86 ± 4	101 ± 0	89 ± 4	23 ± 1	82 ± 9	43 ± 4	102 ± 7
JNK2	88 ± 6	96 ± 3	102 ± 8	99 ± 3	69 ± 3	92 ± 0	93 ± 1	98 ± 1	93 ± 7	24 ± 3	73 ± 5	29 ± 3	97 ± 4
JNK3	87 ± 2	100 ± 4	96 ± 1	90 ± 10	84 ± 0	82 ± 5	92 ± 0	95 ± 0	94 ± 9	47 ± 0	72 ± 5	40 ± 4	96 ± 0
p38α MAPK	96 ± 4	95 ± 5	104 ± 1	95 ± 6	93 ± 6	93 ± 10	89 ± 4	101 ± 3	102 ± 9	110 ± 2	72 ± 8	63 ± 1	82 ± 6
p38β MAPK	94 ± 3	90 ± 2	91 ± 5	78 ± 5	91 ± 1	89 ± 7	84 ± 6	101 ± 2	99 ± 8	106 ± 2	96 ± 5	87 ± 8	102 ± 1
p38γ MAPK	99 ± 2	94 ± 8	98 ± 5	84 ± 2	103 ± 2	82 ± 8	98 ± 4	96 ± 6	79 ± 8	86 ± 1	78 ± 8	46 ± 4	102 ± 5
p38δ MAPK	92 ± 7	98 ± 4	90 ± 2	88 ± 9	86 ± 6	88 ± 3	78 ± 7	91 ± 3	87 ± 1	82 ± 2	68 ± 10	69 ± 5	101 ± 5
ERK5	40 ± 5	70 ± 6	93 ± 2	51 ± 1	19 ± 5	9 ± 1	42 ± 6	56 ± 2	47 ± 2	43 ± 1	48 ± 3	12 ± 5	86 ± 6
RSK1	97 ± 2	74 ± 2	31 ± 5	55 ± 7	85 ± 4	62 ± 3	66 ± 1	91 ± 8	97 ± 5	55 ± 0	71 ± 6	40 ± 1	51 ± 2
RSK2	71 ± 3	71 ± 3	57 ± 6	50 ± 3	105 ± 1	66 ± 7	82 ± 4	92 ± 4	90 ± 6	70 ± 1	89 ± 8	52 ± 6	83 ± 1
PKD1	80 ± 8	79 ± 5	91 ± 4	73 ± 7	83 ± 1	94 ± 8	87 ± 3	98 ± 6	93 ± 11	56 ± 6	102 ± 6	92 ± 9	74 ± 3
PKBα	100 ± 3	95 ± 6	103 ± 6	93 ± 11	92 ± 4	72 ± 7	90 ± 1	88 ± 5	81 ± 7	97 ± 1	78 ± 4	32 ± 3	96 ± 4
PKBβ	83 ± 4	92 ± 3	102 ± 3	95 ± 1	107 ± 1	65 ± 14	101 ± 9	93 ± 1	103 ± 8	92 ± 1	95 ± 7	51 ± 5	90 ± 5
SGK1	75 ± 2	82 ± 1	80 ± 7	80 ± 3	96 ± 6	80 ± 5	75 ± 0	91 ± 4	105 ± 6	28 ± 0	62 ± 3	69 ± 5	57 ± 2
SOK1	90 ± 5	87 ± 3	84 ± 2	47 ± 4	74 ± 1	59 ± 4	86 ± 1	83 ± 3	84 ± 9	85 ± 1	96 ± 4	65 ± 5	98 ± 4
PKA	73 ± 1	79 ± 5	60 ± 6	102 ± 10	93 ± 1	85 ± 9	86 ± 8	79 ± 3	85 ± 2	80 ± 6	112 ± 8	77 ± 5	71 ± 5
ROCK 2	90 ± 4	93 ± 6	86 ± 1	68 ± 5	99 ± 1	67 ± 6	69 ± 6	80 ± 5	59 ± 3	92 ± 2	80 ± 3	32 ± 7	97 ± 5
PRK2	89 ± 2	81 ± 3	61 ± 7	73 ± 3	73 ± 1	37 ± 8	77 ± 7	84 ± 7	35 ± 1	70 ± 5	88 ± 12	58 ± 8	90 ± 5
PKCα	89 ± 2	77 ± 1	93 ± 3	87 ± 2	77 ± 4	92 ± 11	77 ± 2	88 ± 3	67 ± 6	68 ± 2	59 ± 6	80 ± 7	90 ± 6
PKCζ	82 ± 6	83 ± 7	76 ± 6	97 ± 1	84 ± 1	94 ± 7	78 ± 6	84 ± 4	75 ± 10	106 ± 2	86 ± 4	85 ± 12	88 ± 5
PKD1	90 ± 4	85 ± 7	100 ± 4	82 ± 3	81 ± 4	94 ± 6	100 ± 4	54 ± 5	75 ± 2	26 ± 0	108 ± 10	40 ± 11	86 ± 4
MSK1	78 ± 1	89 ± 0	86 ± 1	74 ± 13	94 ± 1	64 ± 4	99 ± 1	82 ± 9	84 ± 8	72 ± 9	89 ± 12	69 ± 5	87 ± 2
MNK1	90 ± 9	92 ± 5	88 ± 4	83 ± 2	10 ± 1	19 ± 3	44 ± 2	91 ± 6	72 ± 4	67 ± 4	68 ± 10	50 ± 8	35 ± 2
MNK2	97 ± 2	98 ± 1	96 ± 2	89 ± 6	50 ± 7	64 ± 1	70 ± 2	92 ± 2	91 ± 3	59 ± 5	70 ± 6	39 ± 4	56 ± 2
MAPKAP-K2	97 ± 7	106 ± 7	83 ± 6	104 ± 8	95 ± 4	80 ± 3	83 ± 4	100 ± 3	97 ± 3	102 ± 2	85 ± 7	54 ± 3	118 ± 2
MAPKAP-K3	86 ± 5	88 ± 1	87 ± 1	103 ± 7	102 ± 1	102 ± 5	95 ± 9	94 ± 3	92 ± 3	103 ± 1	105 ± 8	96 ± 5	104 ± 4
PRAK	83 ± 4	91 ± 2	86 ± 2	86 ± 13	79 ± 2	96 ± 6	85 ± 5	91 ± 7	61 ± 6	58 ± 9	97 ± 8	67 ± 3	96 ± 4
CaMKKα	75 ± 5	76 ± 1	78 ± 4	83 ± 8	43 ± 1	81 ± 9	46 ± 6	82 ± 7	76 ± 2	81 ± 4	2 ± 1	1 ± 1	87 ± 6
CaMKKβ	71 ± 5	89 ± 1	86 ± 7	61 ± 2	8 ± 1	65 ± 4	69 ± 8	80 ± 6	87 ± 1	41 ± 4	91 ± 7	41 ± 4	90 ± 5
CaMK1	104 ± 3	99 ± 1	86 ± 4	76 ± 7	92 ± 7	85 ± 5	73 ± 1	77 ± 4	87 ± 7	73 ± 2	101 ± 2	106 ± 7	95 ± 1
SmMLCK	84 ± 3	86 ± 2	102 ± 7	61 ± 5	81 ± 1	52 ± 4	88 ± 1	87 ± 3	80 ± 7	34 ± 4	90 ± 8	57 ± 5	99 ± 1
PHK	93 ± 1	85 ± 3	58 ± 2	48 ± 7	84 ± 1	6 ± 2	85 ± 7	88 ± 4	103 ± 8	31 ± 0	85 ± 3	62 ± 6	61 ± 3
CHK1	87 ± 5	95 ± 4	77 ± 3	63 ± 1	87 ± 0	93 ± 5	90 ± 5	86 ± 3	69 ± 9	63 ± 3	97 ± 1	95 ± 9	84 ± 6
CHK2	93 ± 4	94 ± 7	93 ± 4	16 ± 0	90 ± 1	95 ± 3	102 ± 8	86 ± 7	93 ± 12	44 ± 5	95 ± 4	64 ± 5	95 ± 2
GSK3β	76 ± 6	79 ± 6	78 ± 4	83 ± 8	43 ± 1	81 ± 9	46 ± 6	82 ± 7	76 ± 2	81 ± 4	2 ± 1	1 ± 1	87 ± 6
CDK2-Cyclin A	4 ± 0	9 ± 1	86 ± 2	87 ± 6	64 ± 2	88 ± 3	76 ± 0	57 ± 8	84 ± 7	50 ± 1	86 ± 6	62 ± 5	81 ± 1
PLK1	91 ± 1	81 ± 3	94 ± 6	83 ± 1	83 ± 2	106 ± 2	98 ± 6	95 ± 7	99 ± 7	80 ± 3	106 ± 7	43 ± 6	91 ± 2
AURORA B	94 ± 6	89 ± 5	7 ± 1	3 ± 0	60 ± 1	40 ± 3	70 ± 6	70 ± 5	50 ± 4	15 ± 1	84 ± 7	57 ± 5	64 ± 4
AURORA C	96 ± 4	85 ± 3	17 ± 1	10 ± 0	68 ± 4	57 ± 7	80 ± 6	91 ± 5	70 ± 2	20 ± 3	70 ± 6	48 ± 7	82 ± 4
AMPK	91 ± 2	89 ± 1	80 ± 6	52 ± 5	18 ± 1	27 ± 4	88 ± 4	86 ± 4	77 ± 10	44 ± 5	82 ± 7	70 ± 4	101 ± 8
MARK3	87 ± 5	89 ± 8	74 ± 3	70 ± 6	109 ± 1	54 ± 3	89 ± 4	90 ± 6	64 ± 5	49 ± 5	86 ± 9	50 ± 7	80 ± 1
BRSK2	79 ± 4	76 ± 6	62 ± 1	53 ± 2	93 ± 4	46 ± 4	90 ± 1	84 ± 4	77 ± 2	44 ± 8	92 ± 7	73 ± 6	39 ± 8
MELK	88 ± 7	72 ± 3	20 ± 1	84 ± 6	77 ± 5	5 ± 0	70 ± 1	79 ± 3	80 ± 5	15 ± 4	96 ± 3	66 ± 7	68 ± 6
CK1δ	76 ± 1	103 ± 5	104 ± 5	57 ± 6	68 ± 0	43 ± 4	92 ± 1	55 ± 5	91 ± 12	15 ± 0	71 ± 7	51 ± 6	30 ± 3
CK2	92 ± 7	99 ± 1	102 ± 1	82 ± 8	10 ± 1	66 ± 5	87 ± 1	87 ± 4	75 ± 6	57 ± 4	63 ± 6	22 ± 3	87 ± 3
DYRK1A	87 ± 5	88 ± 7	91 ± 6	90 ± 7	48 ± 1	12 ± 1	47 ± 2	81 ± 2	41 ± 1	40 ± 1	33 ± 3	9 ± 2	91 ± 6
DYRK2	90 ± 4	91 ± 4	83 ± 2	96 ± 12	34 ± 1	24 ± 1	89 ± 1	78 ± 2	66 ± 4	19 ± 1	12 ± 1	4 ± 2	77 ± 3
DYRK3	97 ± 2	89 ± 4	102 ± 5	95 ± 8	32 ± 1	9 ± 1	50 ± 3	71 ± 2	47 ± 6	30 ± 8	24 ± 3	9 ± 5	67 ± 5
NEK5	85 ± 6	95 ± 1	90 ± 2	87 ± 2	84 ± 1	74 ± 12	85 ± 6	90 ± 5	90 ± 5	78 ± 1	83 ± 10	81 ± 6	91 ± 2
NEK6	92 ± 4	91 ± 5	86 ± 1	98 ± 5	105 ± 1	79 ± 8	83 ± 1	92 ± 4	93 ± 9	102 ± 4	84 ± 9	63 ± 10	93 ± 1
NEK7	87 ± 5	105 ± 5	102 ± 1	99 ± 9	109 ± 1	58 ± 5	103 ± 4	95 ± 5	114 ± 1	112 ± 7	85 ± 7	70 ± 5	103 ± 7
IKKβ	80 ± 3	89 ± 9	84 ± 5	90 ± 8	78 ± 4	98 ± 2	4 ± 0	29 ± 0	28 ± 1	49 ± 3	94 ± 6	86 ± 3	85 ± 3
PIM1	83 ± 5	93 ± 3	88 ± 2	90 ± 7	22 ± 1	80 ± 9	13 ± 1	84 ± 3	40 ± 6	39 ± 3	8 ± 1	5 ± 3	83 ± 5
PIM2	106 ± 4	96 ± 6	99 ± 3	111 ± 12	9 ± 3	109 ± 8	47 ± 1	95 ± 1	55 ± 2	58 ± 2	40 ± 5	7 ± 2	90 ± 1
PIM3	83 ± 9	91 ± 0	77 ± 6	66 ± 7	1 ± 1	85 ± 9	9 ± 1	64 ± 2	25 ± 4	17 ± 0	3 ± 1	2 ± 0	54 ± 2
SRPK1	84 ± 4	79 ± 0	82 ± 6	67 ± 2	60 ± 9	78 ± 4	88 ± 2	92 ± 5	82 ± 3	84 ± 5	96 ± 7	72 ± 1	98 ± 6
MST2	95 ± 5	60 ± 3	54 ± 2	53 ± 3	75 ± 0	96 ± 4	88 ± 3	92 ± 7	69 ± 6	31 ± 4	80 ± 4	86 ± 3	72 ± 6
EP2K	85 ± 8	95 ± 1	93 ± 3	95 ± 9	107 ± 1	114 ± 2	100 ± 2	99 ± 3	113 ± 6	118 ± 1	110 ± 10	90 ± 5	90 ± 2
HIPK2	84 ± 5	99 ± 4	93 ± 4	68 ± 1	35 ± 5	26 ± 1	67 ± 3	83 ± 5	47 ± 2	35 ± 8	29 ± 3	7 ± 1	94 ± 1
HIPK3	90 ± 1	101 ± 2	94 ± 5	95 ± 0	68 ± 8	63 ± 5	89 ± 2	96 ± 9	77 ± 7	56 ± 5	61 ± 4	18 ± 2	106 ± 0
PAK4	90 ± 3	43 ± 0	38 ± 1	79 ± 7	84 ± 1	83 ± 4	93 ± 1	81 ± 4	88 ± 4	60 ± 4	84 ± 11	38 ± 3	76 ± 4
PAK5	74 ± 3	53 ± 3	60 ± 3	88 ± 6	86 ± 1	93 ± 6	87 ± 2	84 ± 2	82 ± 3	82 ± 1	86 ± 11	54 ± 3	87 ± 1
PAK6	80 ± 2	67 ± 5	69 ± 6	87 ± 4	97 ± 1	92 ± 5	101 ± 0	77 ± 8	89 ± 11	72 ± 5	102 ± 3	89 ± 11	79 ± 5
Sec	89 ± 2	14 ± 2	23 ± 3	85 ± 1	102 ± 1	25 ± 2	98 ± 5	90 ± 7	90 ± 6	89 ± 1	79 ± 4	38 ± 3	67 ± 6
Lck	76 ± 2	74 ± 5	10 ± 4	82 ± 5	93 ± 1	20 ± 2	57 ± 3	79 ± 4	70 ± 7	68 ± 2	83 ± 5	68 ± 7	70 ± 6
CSK	88 ± 4	82 ± 2	71 ± 7	93 ± 8	96 ± 5	69 ± 6	83 ± 1	80 ± 0	81 ± 2	65 ± 4	98 ± 4	77 ± 6	67 ± 1
IKKε				51 ± 8		51 ± 7	81 ± 3	93 ± 2	89 ± 4				
TBK1				34 ± 4		45 ± 8	87 ± 1	93 ± 2	93 ± 1				
IKKα							94 ± 1	97 ± 1	95 ± 5				

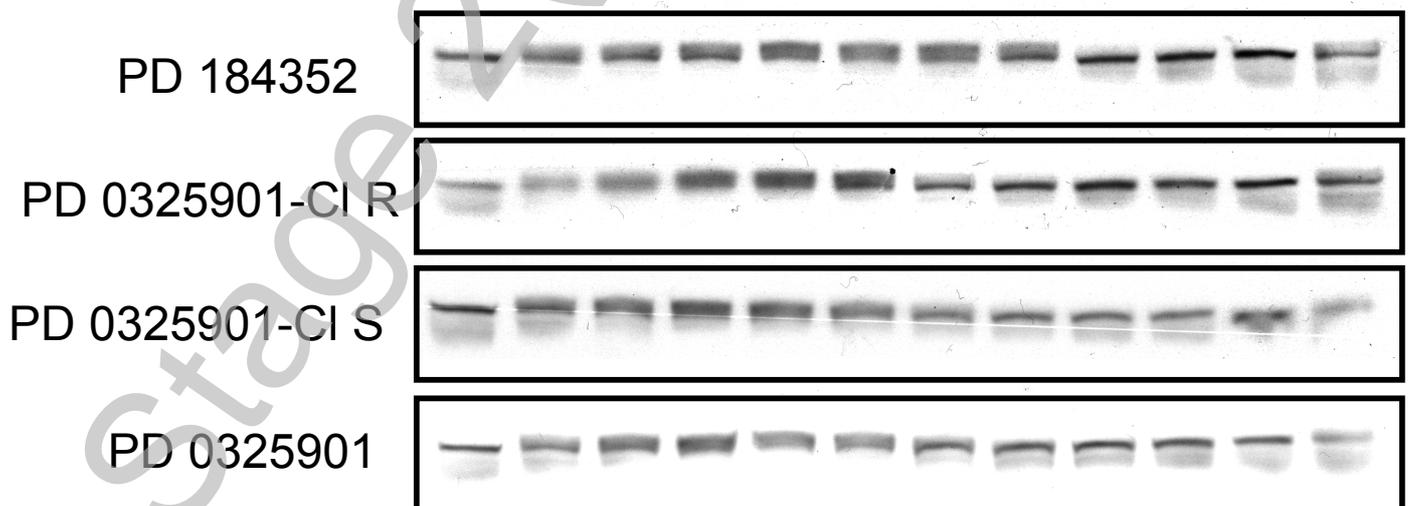
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Figure 1

A - Effect of inhibitors on the activation of ERK1/ERK2



B - Effect of inhibitors on the activation of ERK5



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Figure 2

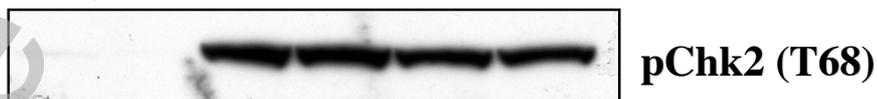
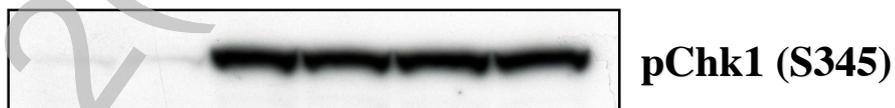
A

PI 103 (0.5 μM)	-	-	-	-	+	+
IGF-1 (50 ng/ml)	-	-	+	+	+	+



B

PI 103 (0.5 μM)	-	-	-	-	+	+
MMS (2mM)	-	-	+	+	+	+



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