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# Design and evaluation of 3-aminopyrazolopyridinone kinase inhibitors inspired by the natural product indirubin

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ABSTRACT

A lead-like kinase inhibitor screening library containing new 3-aminopyrazolopyridinones and closely related compounds was designed that contained hydrogen-bond donor-acceptor motifs and substitution vectors inspired by the natural product kinase inhibitor indirubin. The solubility of the 3-aminopyrazolopyridinone scaffold was more than 1000-fold greater than that of indirubin itself, and solubility was enhanced by reduction of the proportion of lipophilic aryl substituents or the introduction of basic groups. Several components of the library showed kinase inhibitory activity. A subset of diaryl-substituted analogues preferentially inhibited tyrosine kinases with low micromolar activity and good ligand efficiency, and showed cellular antiproliferative activity. The evaluation of the library shows that new, non-natural compounds with relevant biological activity and improved physicochemical properties can be generated from the natural product indirubin, providing compounds that may be useful for kinase inhibitor drug discovery.



## 1. Introduction

Indirubin (**1**, Fig. 1A) is an active component in Danggui Longhui Wan, a Chinese medicine used to treat leukaemia.[1,2] It was isolated from the indigo plant and has been shown to have activity against various types of cancer.[3,4] There are however some gastrointestinal side-effects associated with its use in patients and it has very low solubility, limiting its possible routes of administration and maximum dose.[2,5] Indirubin inhibits a number of protein kinases through competition with the essential co-factor ATP and is thought to exert its action in vivo by this mechanism. It was first shown to have inhibitory activity against CDKs and GSK3β although since then many other kinase targets have been identified for the parent compound, its 3′-oxime or other derivatives.[1,6–12]

Protein kinases are validated targets for a number of diseases, including cancer, and are responsible for the control of numerous cellular processes such as cell cycle progression, apoptosis, growth and metabolism.[13–15] There is therefore ongoing interest in the discovery of new protein kinase inhibitors, of varying specificity and selectivity, for the development of new drugs, and for use as pharmacological tools.[16,17] One approach to making a kinase directed screening library is to use known biologically active molecules, sometimes based on natural products, as a starting point, as successfully demonstrated for purine analogues.[18,19]

Kinase directed libraries have proved useful in the pharmacological studies of kinases, and for hit generation in drug discovery.[17,18,20] There are numerous natural product kinase inhibitors which have been starting points for drug design; for example, the indolocarbazole pan-kinase inhibitor staurosporine,[21,22] the macrolide rapamycin and analogues used in the treatment of cancer,[23,24] the fungal metabolite balanol[25,26] the marine natural product hymenialdisine,[27,28] and the tyrosine kinase inhibitor nakijiquinones.[29,30] Recent examples of natural product-inspired kinase inhibitor libraries include irreversible inhibitors based on simplified analogues of the resorcylic acid lactone hypothemycin.[31] Thus natural products provide a rich resource for kinase inhibitor design.

There have been a number of attempts to improve the activity, selectivity and solubility of indirubin through analogue synthesis. The majority of these have involved making changes to the natural product structure by the addition of small groups around the fused phenyl rings or changing the ketone to an oxime.[32–37] Although some of these compounds have shown improved activity and solubility, the improvement has been limited by the inherently poorly soluble tetracyclic indirubin core. The solubility has also been increased by appending large hydrophilic groups, for example, sugars.[34,38] Although this significantly improves the solubility, the kinase inhibitor potency can be severely reduced, presumably due to an inability of these larger molecules to fit the ATP binding pocket of the protein.

An alternative approach to kinase directed library design based on indirubin would be to remove the parts of the molecule which may not be required for essential binding interactions to protein

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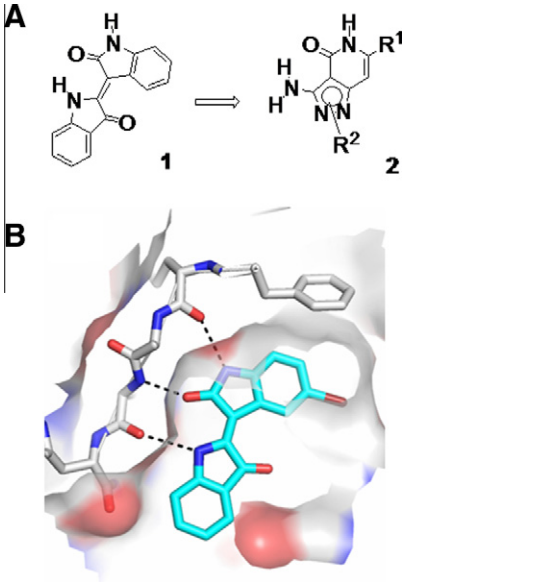



**Figure 1.** (A) Indirubin (**1**) and the 3-aminopyrazolopyridinone scaffold (**2**) share a similar hydrogen-bond donor–acceptor motif; (B) X-ray crystal structure of 5-bromo-indirubin bound to CDK2 (PDB: 2BHE) showing the three hydrogen bonds to the hinge region of the kinase.

kinases and build from a smaller starting point. This also allows the addition of water solubilising groups without overly increasing the molecular weight. With this strategy it is potentially easier to keep the size and lipophilicity of the synthesised compounds within 'drug-like' limits and should lead to better inherent aqueous solubility and membrane permeability.[39,40] However, since radical changes will be made to the natural product structure that serves as inspiration for the library, the likelihood of exactly replicating the biological activity of the natural product is significantly reduced. Thus it is important to evaluate libraries designed in this way to demonstrate that the relevant targeted activity is retained in the modified scaffold.

X-ray crystal structures have been solved for a number of protein kinases with indirubin bound[34,41] and all show the same three heteroatoms to be involved in specific hydrogen bonding within the ATP-binding sites of the proteins (Fig. 1B, shown for 5-bromo-indirubin bound to CDK2). The natural product and analogues bind to the peptide backbone of a linker region ('hinge region') between the two main subdomains of the kinase domain.[42,43] There is an acceptor-donor-acceptor triplet in this region which is often targeted for kinase inhibitor binding.[44,45] Indirubin interacts through the fused pyrrolidin-2-one ring and the NH of the other 5-membered ring, which could be viewed as a vinylagous urea NH. We hypothesised that this core pharmacophore could be reproduced by 3-amino-1*H*-pyrazolo-[4,3-*c*]-pyridin-4(5*H*)-one (3-aminopyrazolopyridinone, **2**, Fig. 1A) a low molecular weight (ca. 150 kDa), planar bicycle which retains the hydrogen bond donor-acceptor motifs of **1** in the same relative positions. As well as potentially mimicking the hydrogen bonding of indirubin, the scaffold **2** could be derivatised to place substituents in similar spaces to the fused phenyl rings of **1** (e.g., $R^1$ and $R^2$, Fig. 1A). In addition, given the caveat that radical changes to the indirubin structure may fail to maintain the original bioactivities, the several hydrogen bond donors-acceptor pairs in the scaffold **2** could allow it to bind to the target enzymes in other orientations, and thus provide opportunities to identify kinase inhibitors unrelated to indirubin from the same library. For example, the 3-aminopyrazole substructure embedded in the scaffold (**2**, $R^2$ = H) represents an alternative and well exemplified hinge-binding motif.[46,47] Simple bicyclic 3-aminopyrazolopyridinones have been reported to provide inhibitors of poly(ADP-ribose) polymerase[48], while isolated reports of activity against kinases for a limited number of compounds with this core have appeared.[49–51] Thus, we undertook the preparation of a library of 3-aminopyrazolopyridinones and closely related compounds[52] and have now evaluated the ability of this library to provide new start points for kinase inhibitor discovery with improved physicochemical properties over the natural product **1** that inspired the design.

## 2. Results and discussion

### 2.1. Library synthesis

Full details of the chemistry for the parallel synthesis of the compounds described here have been described previously.[52] Literature procedures were adapted in the first instance to make the 3-aminopyrazolopyridinone scaffold[53–56] (Fig. 2). Condensation of varied dithioketeneacetals with 2-cyanoacetamide gave intermediate 3-cyano-4-thiomethyl-2-pyridones which were condensed with alkyl- or aryl-substituted hydrazines to generate the 3-aminopyrazolopyridinones **2**.[52] This enabled the synthesis of a large number of analogues with varying substitution on the pyridone and the pyrazole rings. The chemistry was applicable to both aryl and aliphatic ketone starting materials and in total 59 compounds were made with these two positions varied (**2**, $R^1$ and $R^2$).

Synthetic routes were developed to introduce functionality at three additional points around the scaffold, to give compounds with the structures **3**–**5** (Fig. 2).[52] Selective functionalisation of the pendant 3-amino group was effected with electrophilic reagents, for example, acid anhydrides or sulfonyl chlorides (**3**, $R^3 \neq H$). Substituents were introduced to C-7 in the fused pyridone ring (**4**, $R^4 \neq H$) through halogenation followed by palladium-catalysed cross-couplings. Conversion of the pyridone amide functionality to a chloroimidate allowed the introduction of N- or C-linked substituents at C-4 (**5**) by $S_NAr$ or palladium-catalysed reactions, respectively. This synthetic manifold led to a core structure which could be varied at five points, facilitating potential exploration of structure–activity relationships all around the molecule.

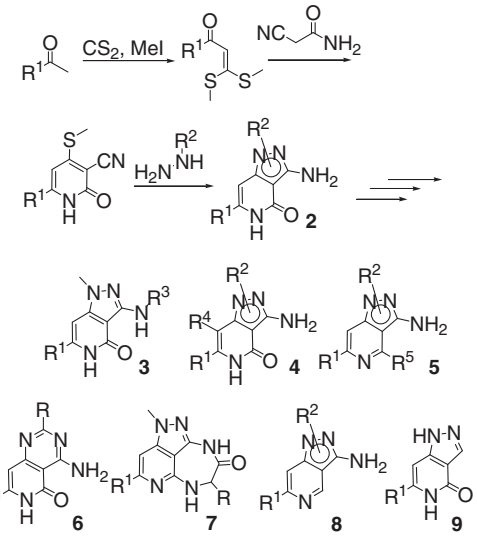



**Figure 2.** Synthetic routes used to prepare the 3-aminopyrazolopyridinones and general structures for the library components.[52]

Hydrazine was replaced with other bifunctional molecules in to access alternative core structures[52] (**6**, Fig. 2). Amidine bis-nucleophiles were used to make ring expanded [6,6]-bicycles **6**, while amino acid methyl esters served as both nucleophile and electrophile in the reaction with chloroimidate derivatives of **2** to form [5,6,7]-tricycles **7**. The removal of some of the possible hydrogen-bond donors and acceptors in the scaffold was also undertaken (**8** and **9**, Fig. 2). These molecules were included as potential control compounds for analysis of early SAR arising from screening of the library, since their activities would inform on the importance of particular hydrogen-bonding interactions. In total, 195 compounds were made in the library and characterised by $^1$H NMR, HRMS and LC–MS to ensure their identity and purity. The synthetic routes have been described fully in a separate publication.[52] A representative sample of 58 compounds from the library, containing members from each of the structural subsets described above, was selected for evaluation of solubility and/or kinase inhibitory activity (Fig. 3).

## 2.2. Physicochemical properties

In selecting the substituents for introduction to the scaffolds **2–9**, it was desirable for the majority of the compounds synthesised to meet guidelines for both drug-like and lead-like calculated physicochemical properties, to increase the probability of adequate solubility and membrane permeability in the screening set. As many kinases are intracellular, it is important that inhibitors can penetrate cell membranes. The guidelines for lead-like properties are more stringent than those for drug-like properties, reflecting the common need for addition of extra atoms during the optimisation of potency and selectivity. As compound sets were selected for synthesis, the proposed structures were evaluated against typical

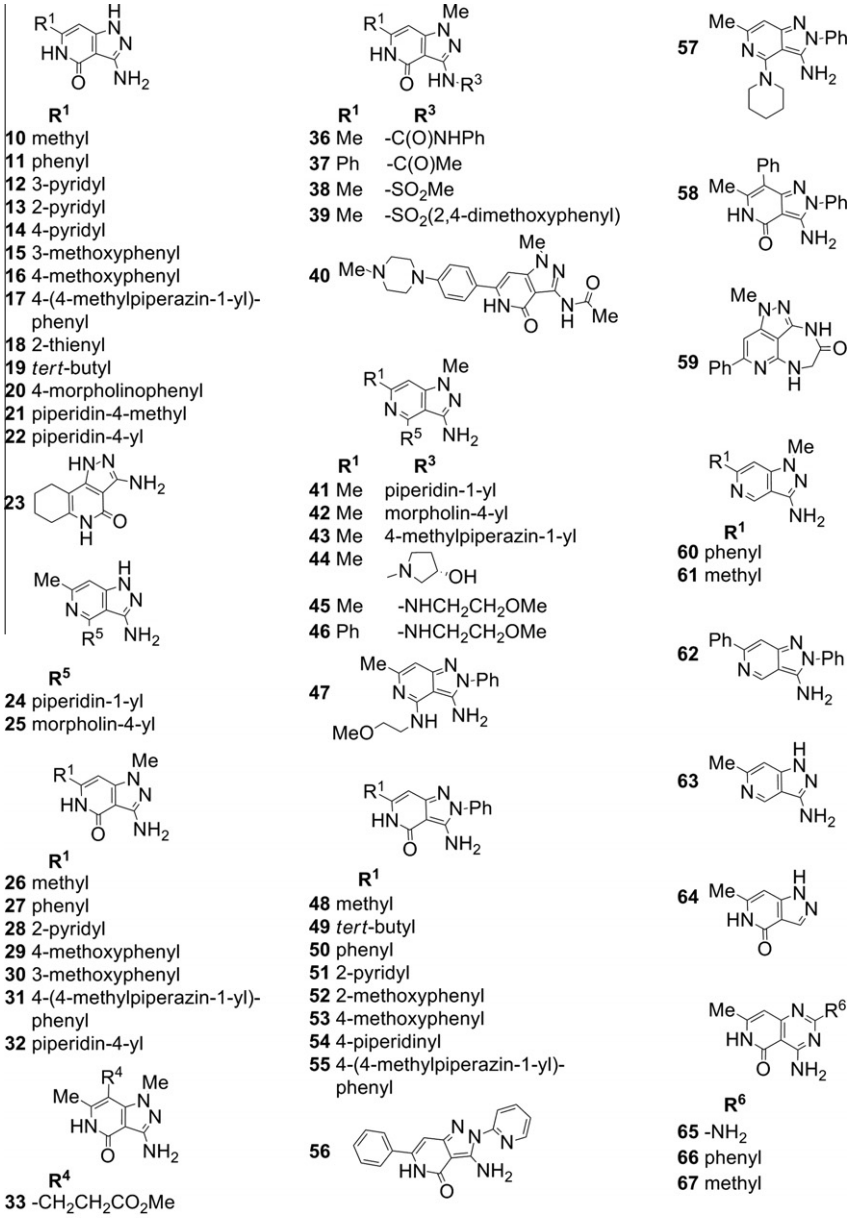



**Figure 3.** Structures of the 58 representative compounds evaluated for aqueous solubility and/or kinase inhibitory activity.

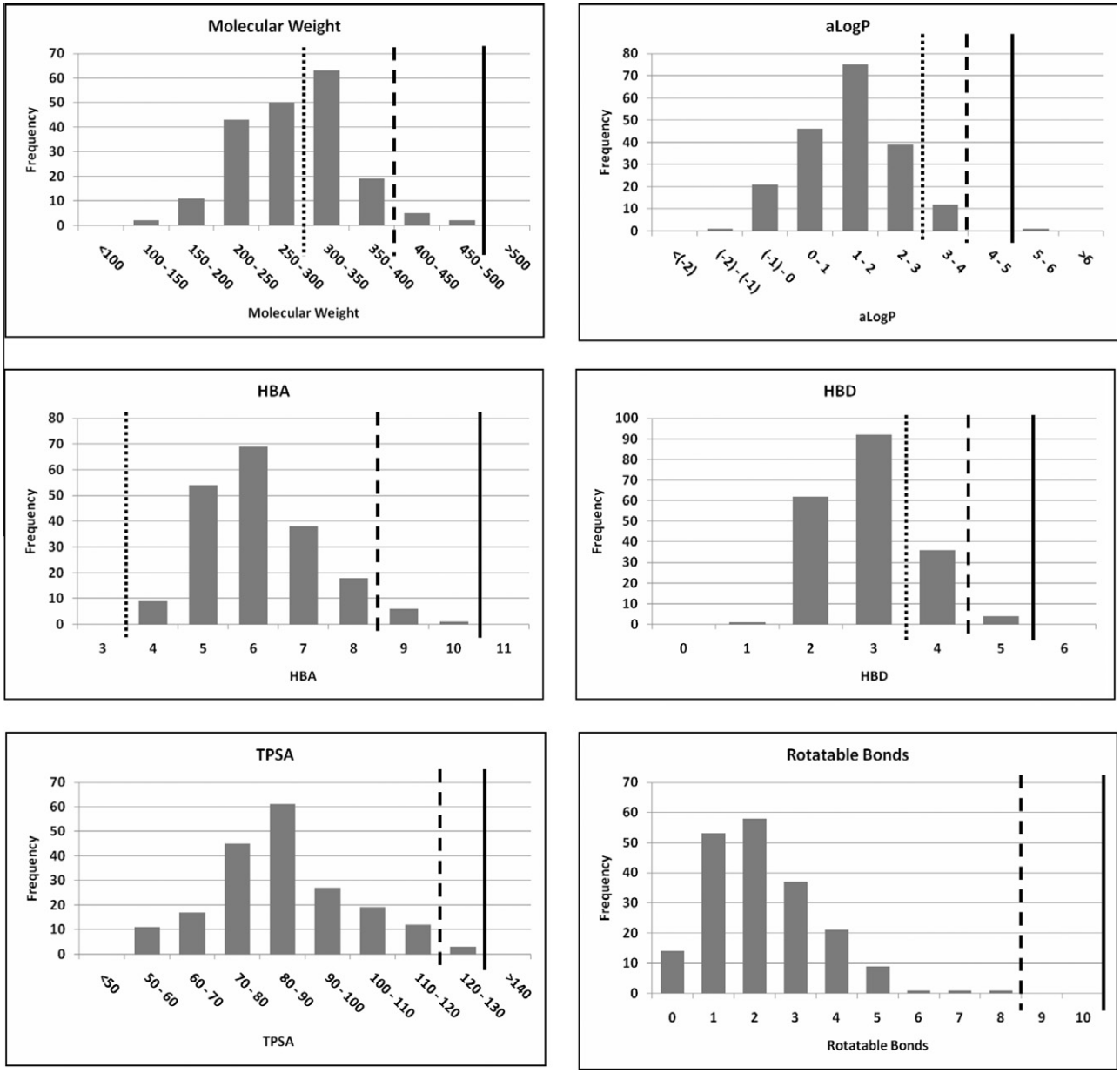



**Figure 4.** Summary of calculated physicochemical properties for 195 compounds synthesised (Molecular weight, *a* Log *P*, number of O and N atoms (HBA), number of OH and NH bonds (HBD), polar surface area (TPSA, Å$^2$) and number of rotatable bonds). Solid lines indicate drug-like maximum guidelines; dashed lines indicate lead-like maximum guidelines; dotted lines indicate fragment maximum guidelines.[17,40]

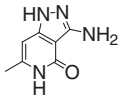
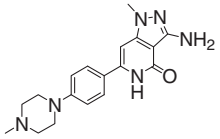
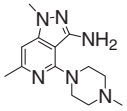
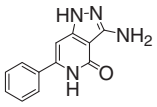
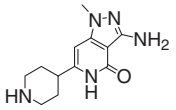
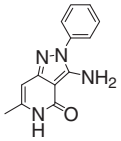
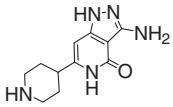
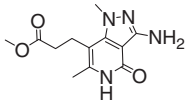
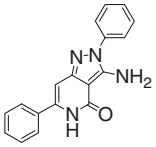
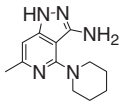
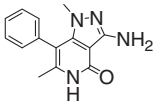
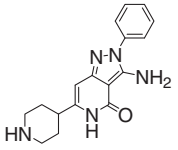
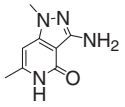
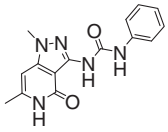
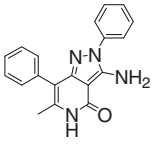
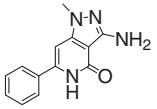
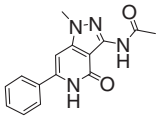
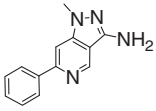
values for calculated drug-like[17,39,40,57,58] and lead-like[17,40,59–62] physicochemical properties. This analysis is summarised for the 195 compounds in the complete library in Figure 4 (drug-like guidelines, solid lines; lead-like guidelines dashed lines). Since a number of the compounds have MW <300 kDa, the library is also compared to proposed calculated physicochemical definitions of fragments[40,63] (Fig. 4, dotted lines). All but one of the compounds fell within drug-like parameters (and this exception had an *a* Log *P* of 5.02, just outside the limit). The majority of the compounds were also within the lead-like parameters proposed as desirable for a biochemical screening library. A significant number of the compounds had MW and *a* Log *P* values consistent with a description of the molecules as fragments. However, the number of potential H-bond accepting N or O atoms was higher than that proposed for fragments.[63] This reflects the high number of heteroatoms in the core, contributed from both the deliberately conserved hinge-binding pharmacophore and the pyrazole ring nitrogens.

## 2.3. Aqueous solubility

Good aqueous solubility is essential for compounds at all stages of the drug discovery process and in many chemical biology experiments, particularly to avoid assay interference due to aggregation.[17,39,64] Aqueous solubility of >10 mg/L has been suggested as a minimum for most drug-like molecules, unless the compounds are particularly potent.[17,39] It has also been shown that there is a tendency for solubility to decrease throughout the drug discovery process.[65] There are a number of methods for in silico prediction of solubility, although it is often challenging to achieve consistent predictivity across diverse structures.[66]

For this library we determined the aqueous solubility of a representative set of compounds (Table 1) using HPLC to measure the concentration of saturated solutions of the compounds. Indirubin (**1**) has been reported to have a solubility of less than 1 mg/L.[34] This is presumed to be due to its rigid, planar structure and its

**Table 1**
Measured aqueous solubility of selected compounds

| No. | Structure | Sol. (mg/L) | No. | Structure | Sol. (mg/L) | No. | Structure | Sol. (mg/L) |
|---|---|---|---|---|---|---|---|---|
| **10** |  | 1635 | **31** |  | 55 | **43** |  | 1164 |
| **11** |  | 24 | **32** |  | >15000 | **48** |  | 69 |
| **22** |  | 2336 | **33** |  | 469 | **50** |  | <1 |
| **24** |  | 794 | **35** |  | 2 | **54** |  | 1426 |
| **26** |  | 1217 | **36** |  | <1 | **58** |  | 7 |
| **27** |  | 76 | **37** |  | 79 | **60** |  | 259 |

ability to form inter- and intra-molecular hydrogen bonds. One approach to reducing the overall planarity was to have pendant rather than fused phenyl rings to make compound **50**, which is one of the closest library members in overall composition to **1**. This made little difference to the solubility which remained less than 1 mg/L. The 7-phenyl analogue **58**, where non-coplanarity of the 7-phenyl group was ensured by a buttressing 6-methyl substituent had only marginally improved solubility (7 mg/L). The unsubstituted pyrazolopyridinone core itself has very high solubility, as exemplified by **10** and **26**, with solubilities of 1635 mg/mL and 1217 mg/mL, respectively. Unsurprisingly, it is the addition of the lipophilic aryl groups which seems to reduce the solubility, confirmed by the mono-aryl analogues **11**, **27**, **37**, **48**, **60** with intermediate but acceptable solubilities of 24–259 mg/L. Decoration of the unsubstituted 3-amino substituent as an aryl urea was also detrimental to solubility (compare **36** to **26**).

Notably, changing the core scaffold from pyridone to pyridine increased solubility (**60** vs **27**), consistent with the increased basicity of pyridine ($pK_{aH}$ 5.2) compared to that of pyridone ($pK_{aH}$ 0.75).[67] Appending moderately basic groups to the less soluble aryl-substituted cores did not improve solubility (compare **27** and **31**). However, by exchanging one of the aryl groups of the poorly soluble diaryl analogue **50** for piperidine, a solubility of 1426 mg/L was attained for compound **54**. When applied to the more soluble analogues such as **27** this modification gave very high solubility, for example, 32 >15000 mg/L.

## 2.4. Kinase screening

A microfluidic mobility shift assay[68] was used to assess the ability of 52 representative compounds from the library to inhibit the activity of a panel of 24 kinases (Table 2 and Fig. 5). The compounds were tested at a single concentration of 30 μM. The test concentration was chosen with respect to the molecular weights and potential ligand efficiencies[69,70] of the library components. Compounds of MW 200–350 kDa, a range which represents most of the library, with $IC_{50}$ ~30 μM or more potent would typically have ligand efficiencies of at least 0.3 kcal $mol^{-1}$ per non-hydrogen atom, a generally acceptable level of activity relative to molecular size to give confidence of progressibility in a drug discovery project.[40,69,70] H-89[71] was included as a control compound in multiple assays. Good reproducibility was observed for the control determinations in the assay format, supporting the use of singlicate initial screening of the library compounds. Subsequently, $IC_{50}$ determinations were carried out on selected compounds. To compare selectivity profiles across this small panel, $S_{(40\%)}$ (Ambit) scores[72] and Gini coefficients[73] were calculated using the singlicate data.[16] The *S* score chosen ($S_{(40\%)}$ at 30 μM) was calculated by dividing the number of kinases against which a compound showed greater than or equal to 40% inhibition at 30 μM by the total number of kinases tested. *S* values closer to 1 indicate decreasing selectivity. The Gini coefficient takes into account the degree of inhibition of all kinases tested and values closer to 1 indicate increased selectivity.

The screening showed that many of the compounds tested did indeed have kinase inhibitory activity. The panel of 24 kinases is a small size for kinome selectivity studies, and therefore the results should be interpreted cautiously.[16,72] Nevertheless, the data from these experiments indicated interesting differences in kinase inhibitory activity between structural subsets within the library. From Table 2, where compounds have been grouped based on structure, it can be seen that compounds which contain an

**Table 2**
Summary of kinase screening (% inhibition at 30 μM); darker colour indicates greater% inhibition[a]

| Entry | MW | S40 | GINI | MAPKAPK2 | AurA | PKCz | RSK1 | PRAK | Erk1 | PKD2 | CK1d | CHK1 | ABL | FYN | LYN | CHK2 | MET | LCK | SRC | GSK3b | Erk2 | PKA | AKT2 | INSR | p38a | AKT1 | MSK1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **10** | 164 | 0.08 | 0.60 | 30 | 23 | 10 | 6 | 8 | 4 | -1 | 34 | 7 | 12 | 2 | 3 | 27 | -8 | 6 | 14 | 18 | 0 | 57 | 8 | -2 | -7 | -3 | 56 |
| **12** | 227 | 0.21 | 0.38 | 40 | 42 | 1 | 11 | 12 | 19 | 26 | 40 | 9 | 37 | 24 | 39 | 27 | 6 | 34 | 46 | 71 | 14 | 35 | 8 | 7 | 3 | 7 | 36 |
| **13** | 227 | 0.42 | 0.33 | 41 | 38 | 3 | 24 | 18 | 11 | 33 | 80 | 29 | 40 | 54 | 49 | 47 | 23 | 56 | 63 | 60 | 12 | 42 | 8 | 34 | 17 | 2 | 35 |
| **14** | 227 | 0.25 | 0.38 | 37 | 43 | 10 | 22 | 13 | 57 | 29 | 61 | 21 | 28 | 28 | 39 | 34 | 10 | 37 | 46 | 95 | 38 | 55 | 4 | 9 | 1 | 2 | 29 |
| **15** | 256 | 0.50 | 0.35 | 56 | 49 | -1 | 20 | 10 | 20 | 17 | 43 | 13 | 44 | 42 | 49 | 52 | 35 | 48 | 62 | 58 | 15 | 48 | 2 | 20 | 10 | 4 | 49 |
| **16** | 256 | 0.50 | 0.42 | 59 | 49 | 7 | 7 | 7 | 13 | 28 | 46 | 14 | 55 | 50 | 56 | 43 | -4 | 54 | 59 | 78 | 12 | 82 | 11 | 6 | 0 | 21 | 62 |
| **17** | 324 | 0.54 | 0.41 | 55 | 51 | 35 | 26 | 11 | 4 | 15 | 49 | 54 | 86 | 76 | 76 | 68 | 18 | 74 | 85 | 42 | -2 | 98 | 6 | 17 | 7 | 9 | 83 |
| **18** | 232 | 0.54 | 0.40 | 58 | 67 | -27 | 35 | 12 | 15 | 5 | 57 | 44 | 63 | 69 | 63 | 59 | 9 | 58 | 74 | 89 | 15 | 79 | 5 | 21 | 6 | 4 | 63 |
| **19** | 206 | 0.29 | 0.52 | 39 | 34 | -9 | 22 | 1 | 12 | -15 | 14 | 12 | 14 | 48 | 40 | 21 | -2 | 40 | 58 | 41 | 4 | 75 | 4 | 14 | 3 | 3 | 65 |
| **20** | 311 | 0.38 | 0.48 | 55 | 51 | -2 | 16 | 11 | 9 | 15 | 24 | 29 | 61 | 62 | 63 | 38 | -1 | 44 | 74 | 41 | -3 | 88 | 7 | 6 | 6 | -1 | 39 |
| **21** | 247 | 0.08 | 0.69 | 35 | 5 | -56 | 11 | 3 | 4 | -6 | 36 | 16 | 7 | -3 | -1 | 7 | -1 | 4 | 4 | 4 | -2 | 71 | 6 | 1 | 3 | 13 | 62 |
| **23** | 204 | 0.00 | 0.62 | 5 | 12 | -5 | -9 | 0 | 5 | -7 | 7 | 17 | 0 | 11 | 12 | 7 | -8 | 7 | 6 | 23 | 6 | 2 | -1 | -3 | -4 | -3 | 1 |
| **24** | 231 | 0.42 | 0.45 | -1 | 84 | 43 | 46 | 10 | 6 | 14 | 93 | 11 | 18 | 53 | 45 | 33 | 18 | 48 | 33 | 55 | 6 | 86 | 11 | 9 | 1 | 36 | 87 |
| **25** | 233 | 0.38 | 0.54 | 1 | 72 | -28 | 30 | 5 | 15 | -11 | 98 | 13 | 24 | 43 | 51 | 23 | 0 | 44 | 45 | 73 | 7 | 71 | 7 | 0 | -3 | 17 | 74 |
| **26** | 178 | 0.00 | 0.65 | 29 | 4 | 0 | 2 | 5 | -2 | 5 | 28 | -8 | 0 | -8 | -13 | 3 | 4 | -1 | 9 | 1 | 8 | -3 | 4 | 3 | 6 | 11 | 4 |
| **27** | 240 | 0.08 | 0.42 | 37 | 14 | 6 | 8 | 11 | 19 | 14 | 43 | 25 | 26 | 26 | 22 | 16 | 0 | 22 | 32 | 66 | 13 | 7 | 4 | 2 | 7 | 8 | 9 |
| **28** | 241 | 0.08 | 0.48 | 36 | 7 | 0 | 9 | 9 | 10 | 17 | 82 | 34 | 27 | 24 | 10 | 25 | 6 | 21 | 18 | 46 | 9 | 5 | 10 | 16 | 1 | 7 | -4 |
| **29** | 270 | 0.33 | 0.42 | 51 | 33 | -3 | 18 | 16 | 28 | 36 | 43 | 36 | 53 | 56 | 49 | 16 | 21 | 46 | 62 | 81 | 16 | 8 | -8 | 5 | 5 | 9 | 9 |
| **30** | 270 | 0.13 | 0.44 | 47 | 17 | 6 | 22 | 6 | 19 | 25 | 43 | 27 | 21 | 23 | 26 | 31 | 0 | 22 | 30 | 61 | 22 | 7 | 1 | 6 | 2 | -5 | 2 |
| **31** | 338 | 0.50 | 0.40 | 45 | 52 | 7 | 14 | 26 | 13 | 34 | 52 | 67 | 80 | 65 | 54 | 53 | 29 | 65 | 80 | 45 | 4 | 81 | -1 | 9 | 3 | 8 | 29 |
| **33** | 264 | 0.00 | 0.40 | 11 | 9 | 14 | -5 | 2 | 10 | 5 | 17 | 2 | 11 | 7 | 6 | -1 | 9 | 9 | 13 | 17 | 5 | 3 | 3 | 3 | -1 | 5 | 7 |
| **34** | 284 | 0.04 | 0.33 | 24 | 31 | 10 | 18 | 13 | 13 | 21 | 36 | 11 | 23 | 20 | 19 | 18 | 18 | 11 | 15 | 58 | 7 | 10 | 25 | 2 | -1 | 13 | 13 |
| **36** | 297 | 0.00 | 0.50 | 0 | 10 | 19 | -11 | 4 | 4 | -4 | 20 | 5 | 15 | 7 | 16 | 2 | 13 | 12 | 17 | 2 | 7 | -6 | 11 | -5 | 7 | 4 | 0 |
| **37** | 282 | 0.04 | 0.49 | 9 | 2 | 28 | 4 | 3 | 3 | 9 | 17 | 4 | 10 | 21 | 13 | 4 | 16 | 8 | 12 | 17 | 2 | -2 | 40 | 5 | 5 | -9 | 3 |
| **38** | 256 | 0.00 | 0.69 | 1 | -8 | 27 | -6 | 0 | 0 | 8 | 5 | 7 | -2 | 11 | 1 | -1 | 18 | 4 | -2 | 13 | 3 | -2 | 16 | -6 | 0 | 1 | 4 |
| **39** | 378 | 0.00 | 0.65 | 2 | -9 | 21 | -2 | 1 | 4 | 14 | 2 | 0 | 3 | 3 | -1 | 6 | 0 | -1 | 2 | 0 | 1 | 3 | 6 | 2 | -4 | 10 | 3 |
| **40** | 380 | 0.00 | 0.48 | 5 | 15 | 6 | 16 | 6 | 3 | 13 | 4 | 16 | 20 | 15 | 8 | 3 | -2 | 10 | 20 | -3 | 4 | 3 | -1 | 2 | 3 | 4 | 0 |
| **41** | 245 | 0.08 | 0.58 | 7 | 41 | -152 | 23 | 17 | 13 | 16 | 79 | -18 | 19 | 8 | 11 | 4 | 7 | 6 | 4 | 8 | 1 | 6 | 0 | -2 | 4 | 6 | 7 |
| **42** | 247 | 0.04 | 0.64 | -2 | 17 | -80 | 1 | 5 | 4 | -4 | 56 | -5 | 15 | 9 | 17 | 3 | 6 | 10 | 7 | 8 | -1 | -2 | 12 | 3 | 2 | -8 | 4 |
| **44** | 247 | 0.00 | 0.62 | 2 | 0 | 19 | -5 | 1 | 2 | 8 | 9 | 7 | -4 | 1 | 5 | -2 | 0 | 1 | 3 | -6 | 2 | 4 | 4 | -2 | 0 | 7 | 4 |
| **45** | 235 | 0.00 | 0.64 | 6 | 15 | -10 | -4 | 5 | 5 | 4 | 16 | 7 | -8 | -1 | 6 | -2 | -5 | 1 | 2 | 1 | 8 | 19 | -3 | 0 | 1 | 6 | 0 |
| **46** | 297 | 0.04 | 0.72 | 2 | 10 | 20 | -3 | 1 | 3 | 1 | 51 | 7 | -7 | 0 | 4 | 8 | -5 | 9 | 3 | 3 | 0 | -3 | -1 | 1 | 2 | 8 | 0 |
| **47** | 297 | 0.00 | 0.65 | 3 | 32 | -123 | 2 | 2 | 3 | -1 | 9 | 0 | 4 | -3 | 6 | 4 | -1 | 0 | 2 | 10 | -1 | 1 | 2 | 1 | 3 | 3 | 3 |
| **48** | 240 | 0.00 | 0.59 | -4 | 6 | -49 | -11 | 5 | 7 | -1 | 38 | -9 | 20 | 22 | 9 | -4 | 9 | 24 | 26 | 9 | 7 | -8 | 1 | 4 | 26 | 5 | 4 |
| **49** | 282 | 0.04 | 0.54 | 6 | 9 | -1 | 5 | 2 | 21 | 15 | 17 | 11 | 13 | 9 | 8 | 5 | 4 | 8 | 7 | 31 | 18 | 1 | 3 | 4 | 79 | 7 | -1 |
| **50** | 302 | 0.29 | 0.58 | 2 | 61 | -163 | 38 | 22 | 20 | 23 | 27 | -42 | 96 | 96 | 96 | -8 | 10 | 90 | 98 | 29 | 12 | -10 | -1 | 2 | 79 | -2 | 13 |
| **51** | 303 | 0.21 | 0.65 | 4 | 30 | 3 | 6 | 2 | 4 | 15 | -1 | 7 | 95 | 96 | 97 | 3 | 8 | 91 | 98 | 11 | 6 | 0 | 8 | 4 | 32 | 4 | 4 |
| **52** | 332 | 0.25 | 0.62 | -1 | 7 | 4 | 3 | 6 | 21 | 20 | 5 | 3 | 86 | 77 | 86 | 16 | 7 | 72 | 87 | 3 | 13 | 6 | 0 | -2 | 68 | 15 | 7 |
| **53** | 332 | 0.21 | 0.63 | 7 | 10 | 0 | 4 | 12 | 8 | 28 | 16 | 5 | 89 | 88 | 90 | -3 | 7 | 75 | 89 | 14 | 3 | -6 | 0 | 0 | 38 | 16 | 6 |
| **55** | 400 | 0.33 | 0.54 | 4 | 66 | 23 | 19 | 4 | 0 | 15 | 25 | 52 | 99 | 99 | 100 | 18 | 26 | 100 | 99 | 12 | 7 | 2 | 10 | 8 | 85 | 3 | 12 |
| **56** | 303 | 0.13 | 0.60 | 6 | 37 | -3 | 5 | -1 | 2 | 16 | 3 | 2 | 33 | 41 | 40 | 18 | 3 | 15 | 37 | 41 | 0 | -3 | 0 | 5 | 7 | 10 | -4 |
| **57** | 307 | 0.00 | 0.44 | 7 | 10 | -96 | 4 | 10 | 4 | 5 | 24 | -1 | 7 | 7 | 8 | 4 | 1 | 12 | 7 | 7 | 6 | 2 | -7 | -2 | 10 | 1 | 10 |
| **58** | 316 | 0.25 | 0.63 | 9 | 4 | 26 | -2 | 6 | 14 | -3 | 15 | -11 | 42 | 62 | 71 | -1 | 8 | 73 | 71 | 16 | 8 | -11 | 4 | 10 | 78 | -8 | 2 |
| **59** | 279 | 0.00 | 0.51 | 8 | 20 | 5 | 0 | 3 | 5 | 17 | 37 | 6 | 4 | 12 | 6 | 5 | 10 | 6 | 22 | 14 | 2 | 0 | 1 | 2 | 6 | 10 | 0 |
| **60** | 224 | 0.04 | 0.58 | 7 | 33 | -23 | 2 | 5 | 9 | 5 | 72 | 1 | 10 | 8 | 7 | 5 | 2 | 11 | 7 | 17 | 4 | 1 | 6 | 9 | 0 | 4 | 4 |
| **61** | 162 | 0.04 | 0.74 | -2 | 3 | -59 | 1 | 9 | 6 | -3 | 52 | 10 | 2 | 2 | 1 | 3 | -1 | 4 | 3 | 6 | 2 | 1 | -2 | -3 | 2 | 0 | -2 |
| **62** | 286 | 0.04 | 0.64 | 1 | 3 | -43 | -3 | 2 | 5 | -11 | 11 | 6 | 19 | 13 | 29 | 7 | 6 | 13 | 9 | 4 | 1 | 4 | 3 | 3 | 55 | -1 | -2 |
| **63** | 148 | 0.04 | 0.54 | 7 | 31 | -275 | 11 | 13 | 8 | 25 | 54 | -25 | 11 | 3 | 8 | 7 | 3 | 6 | 6 | 9 | 4 | 19 | -3 | 5 | 2 | -1 | 13 |
| **64** | 149 | 0.00 | 0.56 | 11 | 13 | -176 | -3 | 1 | 4 | -30 | 18 | 2 | 4 | 4 | 5 | 12 | -2 | 11 | 5 | 13 | 0 | 8 | 1 | 2 | -5 | 2 | 2 |
| **65** | 191 | 0.00 | 0.47 | 26 | 10 | 8 | -4 | 3 | 9 | 8 | 25 | 15 | 19 | 11 | 10 | 16 | 4 | 12 | 12 | 36 | 9 | -1 | 0 | 0 | -1 | 5 | 6 |
| **66** | 252 | 0.17 | 0.52 | 22 | 34 | -17 | 14 | 5 | 10 | 5 | 25 | 3 | 39 | 32 | 23 | 4 | -1 | 45 | 40 | 86 | 11 | 50 | 2 | 5 | 7 | 7 | 15 |
| **67** | 190 | 0.04 | 0.62 | 18 | 3 | 3 | -23 | 1 | 4 | 6 | 26 | 9 | 18 | 8 | 7 | 5 | -3 | 5 | 9 | 52 | 3 | 2 | 2 | 3 | 2 | -2 | 0 |
| **H89** | mean | n/a | n/a | 41 | 71 | 62 | 89 | 37 | 47 | 83 | 50 | 87 | 8 | 13 | 27 | 81 | 21 | 53 | 18 | 10 | 45 | 100 | 82 | 3 | 5 | 96 | 98 |
| | s.d. | n/a | n/a | 5.01 | 6.59 | 8.00 | 4.12 | 9.24 | 5.19 | 7.22 | 5.24 | 3.19 | 5.15 | 2.73 | 5.35 | 2.48 | 7.71 | 7.92 | 3.67 | 5.54 | 3.39 | 0.52 | 3.66 | 6.59 | 2.58 | 2.16 | 0.52 |

[a] $n = 1$ determination except H89 where $n = 3$–4 determinations with standard deviation (SD) shown.

unsubstituted pyrazole (Table 2. **10**, **12**–**21** and **23**) inhibited a large proportion of the kinases on which they were tested. This is as expected, since the unsubstituted pyrazole provides additional possible binding modes to that of the indirubin starting point. Compounds with a pyrazole *N*-methyl substituent (Table 2, **26**–**31**) which removes this additional potential hinge-binding motif often had a reduced spread of activity (e.g., loss of activity vs PKA. MSK1, AurA, SRC).

The *N*-phenyl pyrazoles (Table 2, **48**–**53**, **55** and **56**) also demonstrated interesting patterns of activity. Those with aryl substitution on both the pyrazole and pyridone rings were in fact selective for tyrosine kinases (TKs) over other subfamilies in the kinome (**50**–**53**, **55** and see Fig. 5). The activity of these diaryl-substituted compounds is interesting, as the most likely way in which they can bind to the kinase hinge region is through the three heteroatoms that are positioned similarly to indirubin. Partial removal of this hinge binding motif in **62** led to a loss of activity. Where the C-6 aryl group was replaced by *tert*-butyl, the analogue **49** retained 85% inhibition of p38α at 30 μM, but this was the only significant activity seen (Fig. 5). Where the *N*-phenyl group was replaced by

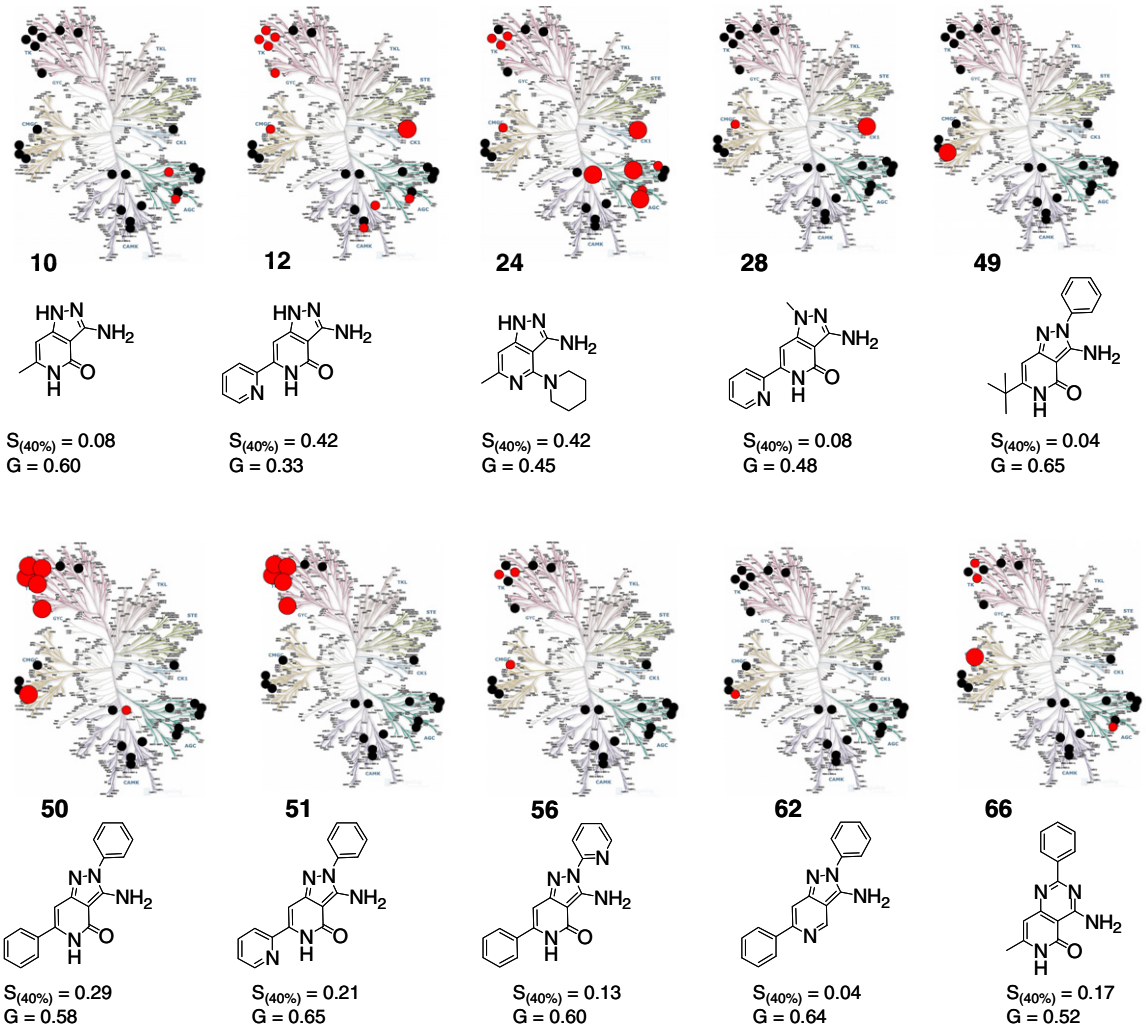



**Figure 5.** Schematic summary of kinase inhibition for selected compounds from the library. Black circle <40% inhibition at 30 μM, small red circle 40–69% inhibition at 30 μM, large red circle 70–100% inhibition at 30 μM. Kinase dendrogram[76] reproduced courtesy of Cell Signaling Technology, Inc. (http://www.cellsignal.com).

2-pyridyl (**56**, Fig. 5) almost all activity was lost. We speculate that this may reflect the formation of an intramolecular hydrogen bond between the 3-amino group and the pyridine, resulting in a more rigid planar conformation that may be incompatible with TK binding (see below).

Compounds with substitution at C-7 (Table 2, **33** and **34**) or on the 3-amino group (Table 2, **36**–**40**) showed little or no inhibitory activity. Interestingly, cyclic amine substitution at C-4 in place of the pyridine carbonyl, although not well tolerated for the *N*-methyl or *N*-phenyl pyrazole analogues (Table 2, **41**, **42**, **44**–**47** and **57**), gave rise to unsubstituted pyrazoles (Table 2, **24** and **25**) that showed a preference for inhibition of kinases in the AGC and CK1 subfamilies. The [6,6]-bicyclic heterocycles (Table 2, **65**–**67**) generally showed little inhibition, although the analogue **66** had some activity on GSK3β, a target of indirubin **1**.

Compounds **24**, **49** and **50** were tested against a larger panel of 85 kinases[74] and showed similar patterns to those seen in the initial panel. Although the 85 kinase panel showed them to be less selective than the 24 kinase panel indicated, compounds **24** and **50** still showed significant preferences for inhibition of CMGC and TK family kinases, respectively. The specificity of **49** for p38 kinase inhibition was also confirmed. The *S* values for **24**, **49** and **50** changed from 0.42, 0.04 and 0.29 to 0.62, 0.08 and 0.34, respectively, in the larger panel, while the Gini coefficients changed from 0.45, 0.65 and 0.58 to 0.35, 0.62 and 0.45, respectively. Thus although the compounds showed quantitatively less selectivity as the testing panel was enlarged, the specificity pattern remained the same.

As the diaryl-substituted compounds **50**–**53** and **55** had a clear pattern of activity, these were investigated further. In each case the compounds inhibited only five of the seven tyrosine kinases in the panel. The two kinases not inhibited are in a different branch of the TK family. It was noted that the gatekeeper residue for the five kinases which were inhibited was threonine, while the gatekeepers for the kinases not inhibited were methionine and leucine. Therefore the slightly larger residues in the latter enzymes may impede the inhibitors' entry into the hydrophobic pocket. Indeed, on docking **50** into the crystal structure of Abl (PDB: 1M52) it was found that the pyrazole *N*-phenyl group was able to access this pocket through a rotated conformation (Fig. 6A), with the hinge-binding motif adopting the desired triplet of hydrogen-bonds taken from indirubin. Compound **50** could be similarly docked into the crystal structures all of the five tyrosine kinases which it inhibited with the same predicted binding pose. Close examination of the predicted binding mode of **50** suggested a hydrogen bond from the 3-$NH_2$ group to the threonine gatekeeper side chain might be possible. This binding mode is consistent with previous observations made from crystal structures of proteins kinases with

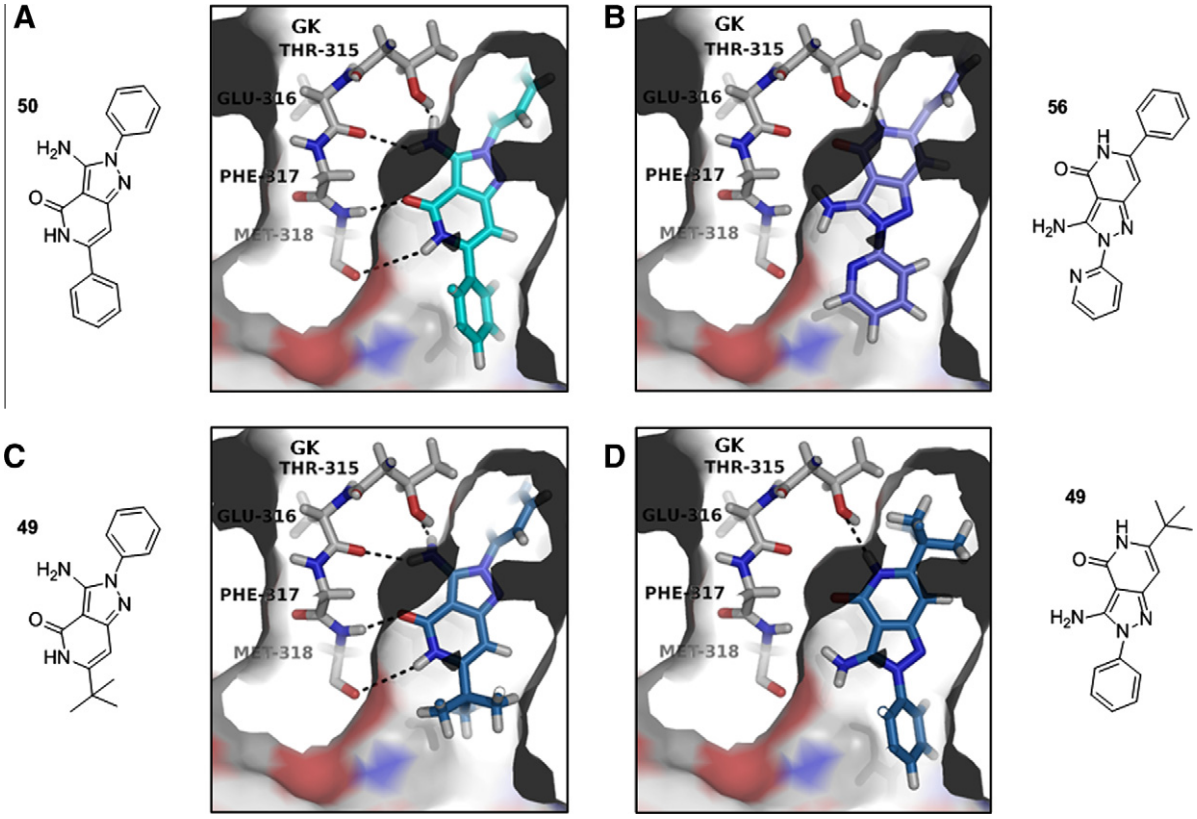



**Figure 6.** Binding poses from docking of (A) **50**, (B) **56** and (C, D) **49** into Abl (PDB: 1M52) using the GOLD[75] algorithm; GK = gatekeeper residue.

**Table 3**
$IC_{50}$ and ligand efficiency values for **31**

| Kinase | $IC_{50}$ (μM) | LE[a] | Kinase | $IC_{50}$ (μM) | LE[a] |
|---|---|---|---|---|---|
| MAPKAPK2 | 35 | 0.25 | LYN | 14 | 0.27 |
| AurA | 33 | 0.25 | CHK2 | 34 | 0.25 |
| CK1δ | 39 | 0.25 | LCK | 18 | 0.27 |
| CHK1 | 24 | 0.26 | SRC | 7.7 | 0.29 |
| Abl | 7.8 | 0.29 | | 38 | 0.25 |
| FYN | 17 | 0.27 | PKA | 5.2 | 0.3 |

[a] Ligand efficiency (kcal $mol^{-1}$ per non-H atom).[69,70]

ATP-competitive ligands bound,[45] where it has been noted that compounds with free $NH_2$ groups tend to bind with the amine towards the inside of the pocket, close to the gatekeeper residue.

Compounds **56** and **49** which have a similar structure to **50**, but little or no TK inhibitory activity, were also docked. When **56** was docked into Abl (Fig. 6B) the binding mode observed for **50** was not obtained. Instead, the scaffold was rotated by 180° to allow the more freely rotatable C-6 aryl group to occupy the hydrophobic pocket. The 2-pyridyl group, conformationally restrained by intramolecular hydrogen bonding, was directed into solvent. This binding pose abolishes hydrogen bonding to the hinge region, and is therefore unlikely to contribute to significant affinity. On docking the C-6 alkyl substituted compound **49** into Abl, two distinct poses were predicted; one similar to that seen for **50** and the other to that of **56** (Fig. 6C). However, although **49** was predicted to be able attain a productive binding pose similar to **50**, no TK activity was seen. The presence of the second aryl substituent, directed towards the solvent-exposed surface, therefore appears to be important for TK inhibition with these compounds.

**Table 4**
$IC_{50}$ and ligand efficiency values for **50**

| Kinase | $IC_{50}$ (μM) | LE[a] | Kinase | $IC_{50}$ (μM) | LE[a] |
|---|---|---|---|---|---|
| Abl | 1.5 | 0.35 | LCK | 4.8 | 0.32 |
| FYN | 1.3 | 0.35 | SRC | 0.87 | 0.37 |
| LYN | 1.4 | 0.36 | P38α | 8.7 | 0.31 |

[a] Ligand efficiency (kcal $mol^{-1}$ per non-H atom).[69,70]

$IC_{50}$s were determined for **31** and **50** using the mobility shift assay. Compound **31** had been shown to inhibit approximately half of the 24 kinases against which it was tested by greater than 40% at 30 μM. For this set of compounds the (1-methylpiperizin-4-yl)phenyl moiety seemed to confer promiscuity as all compounds with this motif (**17**, **31** and **55**) inhibited a large proportion of the kinases against which they were tested. For **31**, moderate potency and ligand efficiency was observed, with $IC_{50}$ values ranged from 5 to 39 μM (Table 3) although partial $IC_{50}$ curves could be seen for some other kinases. Compound **50** was a TK selective inhibitor and showed low micromolar activity on five of the seven tyrosine kinases tested (0.87–4.8 μM, Table 4). Ligand efficiency values indicated that as well as better specificity, compound **50** attains efficient binding (>0.3 kcal $mol^{-1}$ per non-H atom), consistent with potential for elaboration of the molecule to a potent inhibitor while remaining within the drug-like range of MW.[69,70]

Selected compounds were also tested for growth inhibitory activity against MCF7 human breast cancer cells. Many of the compounds showed activity in the region of 40–60 μM, with the diaryl substituted compounds **51** and **50** having sub-micromolar $GI_{50}$s (0.21 μM and 35% at 0.039 μM, respectively). Fifteen out of the eighteen compounds tested showed some antiproliferative activity at 100 μM. Although the activity seen may not be due to kinase inhibitory activity it does suggest good prospects for both cell membrane permeability and cellular activity with this chemical scaffold.

## 3. Conclusions

A screening library containing new 3-aminopyrazolopyridinones and closely related compounds was made that contained hydrogen-bond donor-acceptor motifs and substitution vectors inspired by the natural product kinase inhibitor indirubin. Parallel synthesis was developed for the routes where possible and 195 compounds were made. The substituents decorating the core heterocycles were chosen to generate a library largely consistent with emerging guidelines for the lead-likeness of screening libraries.

The calculated physicochemical properties of the library were mainly lead-like and many of the compounds could also be defined as fragments. The solubility of the 3-aminopyrazolopyridinone scaffold was more than 1000-fold greater than that of indirubin itself, addressing a pharmaceutical liability of the natural product. Although some library components still had low solubility, this could be overcome by varying the substitution, in particular to reduce the proportion of lipophilic aryl substituents or to introduce basicity within the core.

A substantial representative selection of the library was evaluated against a small panel of human kinases and inhibitory activity was shown for a number of the compounds. Some structure-selectivity relationships could be discerned from this screening. This could be tested further with a larger panel of kinases. A subset of diaryl-substituted analogues preferentially inhibited tyrosine kinases with low micromolar activity and good ligand efficiency, which could be rationalised through in silico modelling of the binding modes of the compounds to kinase ATP sites. Other analogues showed preferences for inhibition of other subsets of kinases. Sub-micromolar antiproliferative activity was seen for diaryl-substituted compounds in the MCF7 breast cancer cell line with other compounds having $GI_{50}$s of 40–60 μM.

This data shows that compounds with relevant biological activity and improved physicochemical properties can be generated using the binding mode of the natural product indirubin as an inspiration for the design of new, non-natural compounds. Although most of the new compounds contain a conserved pharmacophore derived from the natural product, there is considerable structural and physicochemical property variation between the 3-aminopyrazolopyridinone core and indirubin. As a consequence, the 3-aminopyrazolopyridinones do not reproduce the distinct kinase activity pattern or potencies of the natural product itself. However, the compounds may be interesting for future drug discovery endeavours as they have good kinase ligand efficiencies, and flexible chemistry has been developed to enable diverse substitutions at five points around the core.

## 4. Experimental

### 4.1. Synthetic chemistry and compound characterisation

Compounds were prepared using the synthetic routes previously reported.[52] All library compounds were evaluated for identity by $^1$H NMR and HRMS, and for purity by HPLC and $^1$H NMR. $^1$H NMR spectra were recorded at 500 MHz on a Bruker AMX500 spectrometer using an internal deuterium lock. Chemical shifts were measured in parts per million (ppm) relative to tetramethylsilane ($\delta = 0$) using the following internal references for residual protons in the solvent: $CDCl_3$ ($\delta_H$ 7.26), $CD_3OD$ ($\delta_H$ 3.32), $(CD_3)_2SO$ ($\delta_H$ 2.50) and $(CD_3)_2CO$ ($\delta_H$ 2.05). Data is presented as: chemical shift, integration, multiplicity, coupling constant ($J$) in Hz and interpretation.

Combined HPLC–MS analyses were recorded using a Waters Alliance 2795 separations module and Waters/Micromass LCT mass detector with electrospray ionisation (+ve ion mode). The molecular weight scan range was 50–1000. HPLC was performed at a temperature of 22 °C with gradient elutions of 10–90% MeOH/0.1% aqueous formic acid using the following columns, run times and flow rates: Phenomenex Gemini C18, 30 mm × 4.6 mm i.d. (6 min, 1 mL/min) or Merck Chromolith SpeedROD RP-18e 50 mm × 4.6 mm i.d. (3.5 min, 2 mL/min). Compounds were detected at 254 nm using a Waters 2487 dual absorbance detector. HRMS analyses were performed on an Agilent 6210 TOF mass spectrometer.

### 4.2. Kinase inhibition

Compounds were tested by microfluidic mobility shift assay[68] using a LabChip® EZReader II from Caliper Life Sciences and ProfilerPro kinase selectivity assay kits from the same supplier. Buffers were added using a Thermo Multidrop Combi and all other liquid transfers were carried out using a PerkinElmer Evolution P3 robotic liquid handling system to eliminate variation due to the time needed to transfer all reagents. Test compounds were dissolved in DMSO to give 1 mM solutions which were subsequently diluted to give 30 μM under final assay conditions. Appropriate fluorescently tagged peptides were used for each kinase and assays were run at apparent Km ATP for each protein. Positive controls contained no inhibitor and negative controls contained no ATP. Phosphorylated and unphosphorylated peptides were separated by electrophoresis, both were detected and the percentage conversion was calculated as a ratio of the area under the phosphorylated peptide peak relative to the sum of both peaks.

Compounds were tested on a standard set of 24 kinases (ProfilerPro Plate 1: Abl, AKT1, AKT2, AurA, CHK1, CHK2, CK1δ, Erk1, Erk2, FYN, GSK3β, INSR, LCK, LYN, MAPKAP2, MET, MSK1, p38α, PKAcα, PKC, PKD2, PRAK/MAPKAP5, RSK1, SRC). In each case H89,[71] a broad spectrum kinase inhibitor with some preference for AGC kinases, was used as a positive control to allow comparisons between plates. For calculation of Gini coefficients, negative percentage inhibitions were treated as zero values.

### 4.3. Docking studies

Docking was carried out using the GOLD program.[75] Crystal structures were taken from the RCSB Protein Data Bank, hydrogens were added and water molecules and co-crystallised ligand were removed. Test compounds were energy minimised using MM2 or MMFF94 force fields and docked into the protein. Compounds were docked into a 10 Å radius sphere, the centre of which was defined by the original co-crystallised ligand. The program was allowed to dock the compounds until three similar binding modes (positioned within 1.5 Å) were identified.

## Conflicts of Interest

The Institute of Cancer Research is an academic reference centre for the Caliper EZReader microfluidic assay platform. I.C. has received consumables grant funding from Caliper Life Sciences Inc.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.069.

## References and notes


1. Hoessel, R.; Leclerc, S.; Endicott, J. A.; Noble, M. E. M.; Lawrie, A.; Tunnah, P.; Leost, M.; Damiens, E.; Marie, D.; Marko, D.; Niederberger, E.; Tang, W.; Eisenbrand, G.; Meijer, L. *Nat. Cell Biol.* **1999**, *1*, 60–67.
2. Xiao, Z.; Hao, Y.; Liu, B.; Qian, L. *Leuk. Lymphoma* **2002**, *43*, 1763–1768.
3. Kim, S.-A.; Kim, Y.-C.; Kim, S.-W.; Lee, S.-H.; Min, J.-J.; Ahn, S.-G.; Yoon, J.-H. *Clin. Cancer Res.* **2007**, *13*, 253–259.
4. Lee, J.-W.; Moon, M. J.; Min, H.-Y.; Chung, H.-J.; Park, E.-J.; Park, H. J.; Hong, J.-Y.; Kim, Y.-C.; Lee, S. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3948–3952.
5. Eisenbrand, G.; Hippe, F.; Jakobs, S.; Muehlbeyer, S. J. *Cancer Res. Clin. Oncol.* **2004**, *130*, 627–635.
6. Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. *Biochem. J.* **2003**, *371*, 199–204.
7. Damiens, E.; Baratte, B.; Marie, D.; Eisenbrand, G.; Meijer, L. *Oncogene* **2001**, *20*, 3786–3797.
8. Jakobs, S.; Merz, K. H.; Vatter, S.; Eisenbrand, G. *Int. J. Clin. Pharmacol. Ther.* **2005**, *43*, 592–594.
9. Meijer, L.; Skaltsounis, A.-L.; Magiatis, P.; Polychronopoulos, P.; Knockaert, M.; Leost, M.; Ryan, X. P.; Vonica, C. A.; Brivanlou, A.; Dajani, R.; Crovace, C.; Tarricone, C.; Musacchio, A.; Roe, S. M.; Pearl, L.; Greengard, P. *Chem. Biol.* **2003**, *10*, 1255–1266.
10. Myrianthopoulos, V.; Magiatis, P.; Ferandin, Y.; Skaltsounis, A.-L.; Meijer, L.; Mikros, E. *J. Med. Chem.* **2007**, *50*, 4027–4037.
11. Ribas, J.; Bettayeb, K.; Ferandin, Y.; Knockaert, M.; Garrofe-Ochoa, X.; Totzke, F.; Schaechtele, C.; Mester, J.; Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A. L.; Boix, J.; Meijer, L. *Oncogene* **2006**, *25*, 6304–6318.
12. Zhen, Y.; Sorensen, V.; Jin, Y.; Suo, Z.; Wiedlocha, A. *Oncogene* **2007**, *26*, 6372–6385.
13. Cohen, P. *Nat. Rev. Drug Disc.* **2002**, *1*, 309–315.
14. Scapin, G. *Curr. Drug Targets* **2006**, *7*, 1443–1454.
15. Zhang, J.; Yang, P. L.; Gray, N. S. *Nat. Rev. Cancer* **2009**, *9*, 28–39.
16. Smyth, L. A.; Collins, I. *J. Chem. Biol.* **2009**, *2*, 131–151.
17. Workman, P.; Collins, I. *Chem. Biol.* **2010**, *17*, 561–577.
18. Chang, Y.-T.; Gray, N. S.; Rosania, G. R.; Sutherlin, D. P.; Kwon, S.; Norman, T. C.; Sarohia, R.; Leost, M.; Meijer, L.; Schultz, P. G. *Chem. Biol.* **1999**, *6*, 361–375.
19. Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P. J.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. *Eur. J. Biochem.* **1997**, *243*, 527–536.
20. Collins, I.; Workman, P. *Curr. Signal Transduction Ther.* **2006**, *1*, 13–23.
21. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282.
22. Gani, O. A. B. S. M.; Engh, R. A. *Nat. Prod. Rep.* **2010**, *27*, 489–498.
23. Albers, M. W.; Williams, R. T.; Brown, E. J.; Tanaka, A.; Hall, F. L.; Schreiber, S. L. *J. Biol. Chem.* **1993**, *268*, 22825–22829.
24. Dancey, J. *Nat. Rev. Clin. Oncol.* **2010**, *7*, 209–219.
25. Kulanthaivel, P.; Hallock, Y. F.; Boros, C.; Hamilton, S. M.; Janzen, W. P.; Ballas, L. M.; Loomis, C. R.; Jiang, J. B.; Katz, B.; Steiner, J. R.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 6452–6453.
26. Pande, V.; Ramos, M. J.; Gago, F. *Anticancer Agents Med. Chem.* **2008**, *8*, 638–645.
27. Supriyono, A.; Schwarz, B.; Wray, V.; Witte, L.; Müller, W. E.; van Soest, R.; Sumaryono, W.; Proksch, P. *Z. Naturforsch. C.* **1995**, *50*, 669–674.
28. Nguyen, T. N.; Tepe, J. J. *Curr. Med. Chem.* **2009**, *16*, 3122–3143.
29. Kobayashi, J.; Madono, T.; Shigemori, H. *Tetrahedron* **1995**, *51*, 10867–10874.
30. Kissau, L.; Stahl, P.; Mazitschek, R.; Giannis, A.; Waldmann, H. *J. Med. Chem.* **2003**, *46*, 2917–2931.
31. Barluenga, S.; Jogireddy, R.; Koripelly, G. K.; Winssinger, N. *ChemBioChem* **2010**, *11*, 1692–1699.
32. Beauchard, A.; Ferandin, Y.; Frere, S.; Lozach, O.; Blairvacq, M.; Meijer, L.; Thiery, V.; Besson, T. *Bioorg. Med. Chem.* **2006**, *14*, 6434–6443.
33. Ferandin, Y.; Bettayeb, K.; Kritsanida, M.; Lozach, O.; Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.-L.; Meijer, L. *J. Med. Chem.* **2006**, *49*, 4638–4649.
34. Jautelat, R.; Brumby, T.; Schaefer, M.; Briem, H.; Eisenbrand, G.; Schwahn, S.; Krueger, M.; Luecking, U.; Prien, O.; Siemeister, G. *ChemBioChem* **2005**, *6*, 531–540.
35. Li, C.; Go, Y.; Mao, Z.; Koyano, K.; Kai, Y.; Kanehisa, N.; Zhu, Q.; Zhou, Z.; Wu, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1621–1627.
36. Moon, M. J.; Lee, S. K.; Lee, J.-W.; Song, W. K.; Kim, S. W.; Kim, J. I.; Cho, C.; Choi, S. J.; Kim, Y.-C. *Bioorg. Med. Chem.* **2006**, *14*, 237–246.
37. Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.-L.; Myrianthopoulos, V.; Mikros, E.; Tarricone, A.; Musacchio, A.; Roe, S. M.; Pearl, L.; Leost, M.; Greengard, P.; Meijer, L. *J. Med. Chem.* **2004**, *47*, 935–946.
38. Libnow, S.; Hein, M.; Michalik, D.; Langer, P. *Tetrahedron Lett.* **2006**, *47*, 6907–6909.
39. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.
40. Collins, I.; Workman, P. *Nat. Chem. Biol.* **2006**, *2*, 689–700.
41. Mapelli, M.; Massimiliano, L.; Crovace, C.; Seeliger, M. A.; Tsai, L.-H.; Meijer, L.; Musacchio, A. *J. Med. Chem.* **2005**, *48*, 671–679.
42. Cherry, M.; Williams, D. H. *Curr. Med. Chem.* **2004**, *11*, 663–673.
43. Johnson, L. N.; Noble, M. E. M.; Owen, D. J. *Cell* **1996**, *85*, 149.
44. Noble, M. E. M.; Endicott, J. A.; Johnson, L. N. *Science* **2004**, *303*, 1800–1805.
45. Ghose, A. K.; Herbertz, T.; Pippin, D. A.; Salvino, J. M.; Mallamo, J. P. *J. Med. Chem.* **2008**, *51*, 5149–5171.
46. Lee, J.; Choi, H.; Kim, K. H.; Jeong, S.; Park, J. W.; Baek, C. S.; Lee, S. H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2292–2295.
47. Antonysamy, S.; Hirst, G.; Park, F.; Sprengeler, P.; Stappenbeck, F.; Steensma, R.; Wilson, M.; Wong, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 279–282.
48. Fujio, M; Satoh, H.; Numata, A.; Takanashi, S.; Egi, Y.; Tatsumi, R. Int. Pat. Appl. WO2002094790, 2002; *Chem. Abs.* **2002**, *138*, 4615.
49. Ronan, B.; Tabart, M.; Halley, F.; Bacque, E.; Souaille, C.; Ugolini, A.; Viviani, F. Int. Pat. Appl. WO 2006077319, 2006; *Chem. Abs.* **2006**, *145*, 167240.
50. Cao, S. X.; Feng, J.; Gwaltney, S. L.; Hosfield, D. J.; Skene, R. J.; Stafford, J. A.; Tang, M. U.S. Pat. Appl. US 20070281942, 2007; *Chem. Abs.* **2007**, *148*, 33724.
51. Mciver, E. G.; Smiljanic, E.; Harding, D. J.; Hough, J. Int. Pat. Appl. WO 2010106333, 2010; Chem *Abs.* **2010**, *153*, 56669.
52. Smyth, L. A.; Matthews, T. P.; Horton, P. N.; Michael, B.; Hursthouse, M. B.; Collins, I. *Tetrahedron* **2010**, *66*, 2843–2854.
53. Kumar, A.; Ila, H.; Junjappa, H. *J. Chem. Soc., Perkin Trans. 1* **1978**, 857.
54. Rastogi, R. R.; Kumar, A.; Ila, H.; Junjappa, H. *J. Chem. Soc., Perkin Trans. 1* **1978**, 549.
55. Thuillier, A.; Vialle, J. *Bull. Soc. Chim. Fr.* **1959**, 1398–1401.
56. Tominaga, Y.; Kawabe, M.; Hosomi, A. J. *Heterocycl. Chem.* **1987**, *24*, 1325.
57. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615–2623.
58. Kubinyi, H. *Nat. Rev. Drug Disc.* **2003**, *2*, 665–668.
59. Davis, A. M.; Keelin, D. J.; Steele, J.; Tomkinson, N. P.; Tinker, A. C. *Curr. Top. Med. Chem.* **2005**, *5*, 421–439.
60. Lumley, J. A. *QSAR Comb. Sci.* **2005**, *24*, 1066–1075.
61. Oprea, T. I.; Davis, A. M.; Teague, S. J.; Leeson, P. D. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1308–1315.
62. Teague, S. J.; Davis, A. M.; Leeson, P. D.; Oprea, T. *Angew. Chem, Int. Ed.* **1999**, *38*, 3743–3748.
63. Congreve, M.; Carr, R.; Murray, C.; Jhoti, H. *Drug Discovery Today* **2003**, *8*, 876–877.
64. McGovern, S. L.; Caselli, E.; Grigorieff, N.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 1712–1722.
65. Oprea, T. I. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 325–334.
66. Dearden, J. C. *Expert Opin. Drug Discovery* **2006**, *1*, 31–52.
67. Hilal, S. H.; Karickhoff, S. W.; Carreira, L. A. *Quant. Struct.-Act. Relat.* **1995**, *14*, 348–355.
68. Card, A.; Caldwell, C.; Min, H.; Lokchander, B.; Hualin, X.; Sciabola, S.; Kamath, A. V.; Clugston, S. L.; Tschantz, W. R.; Leyu, W.; Moshinsky, D. J. *J. Biomol. Screen.* **2009**, *14*, 31–42.
69. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discov. Today* **2004**, *9*, 430–431.
70. Abad-Zapatero, C.; Metz, J. T. *Drug Discov. Today* **2005**, *10*, 464–469.
71. Chijiwa, T.; Mishima, A.; Hagiwara, M.; Sano, M.; Hayashi, K.; Inoue, T.; Naito, K.; Toshioka, T.; Hidaka, H. *J. Biol. Chem.* **1990**, *265*, 5267–5272.
72. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127–132.
73. Graczyk, P. P. *J. Med. Chem.* **2007**, *50*, 5773–5779.
74. National Centre for Protein Kinase Profiling, Dundee University, UK.
75. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727–748.
76. Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2002**, *298*, 1912–1916. 1933–1934.