Original article

Single step synthesis of new fused pyrimidine derivatives and their evaluation as potent Aurora-A kinase inhibitors

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Structure–activity relationship (SAR)
Molecular modeling

A B S T R A C T

A simple, facile, efficient and one pot three-component procedure for the synthesis of pyrazolo[1,5-a]pyrimidines, triazolo[1,5-a]pyrimidines and pyrimido[1,2-a]benzimidazoles ring systems incorporating phenylsulfonyl moiety was developed via the reaction of 1-aryl-2-(phenylsulfonyl)ethanone derivatives 1a–d with the appropriate heterocyclic amine and triethyl orthoformate and evaluated as Aurora-A kinase inhibitors. The cytotoxic activity of the newly synthesized compounds against HST116 colon tumor cell line was investigated. 2,7-Diphenyl-6-(phenylsulfonyl)pyrazolo[1,5-a]pyrimidine (4b) and its p-methoxy analogue 4c were found to be equipotent to Doxorubicin as a reference drug. Molecular modeling study was carried out in order to rationalize the in vitro anti-tumor results.

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1. Introduction

Colon cancer is one of the most common tumors that occurs in the western world and usually ranks high in incidence and mortality among new malignant cases [1]; there is a strong correlation between aurora kinase expressions and colon carcinoma. The expression of aurora kinase protein was found to be related to grade of tumor, p16 expression, and telomerase activity. These findings are important to select patients for clinical trials of Aurora kinase inhibitors [2–5].

Aurora-A is a member of a family of mitotic serine/threonine kinases which includes also types B and C. It is implicated with important processes during mitosis and meiosis whose proper function is integral for healthy cell proliferation. Its activity peaks have been observed during the G2 phase to M phase transition in the cell cycle [6]. Aurora-A kinase plays an important role in mitosis. It is associated with centrosome maturation and separation and thereby regulates spindle assembly and stability [7]. Overexpression or deregulation of Aurora-A has been associated with a high occurrence of cancer [8]. Deregulation of Aurora-A may lead to cancer because it is required for the completion of cytokinesis. If the cell begins mitosis, duplicates its DNA, but is then unable to divide into two separate cells, it becomes an aneuploid (i.e. it contains more chromosomes than normal). Aneuploidy is a trait of many cancerous tumors [9]. Among compounds that can be used as Aurora kinase inhibitors, pyrazolo[1,5-a]pyrimidine occupy an important position [10].

In continuation of our recent work aiming at the synthesis of a variety of heterocyclic systems with remarkable biological importance [11–26], we report here on the utility of ϒ-keto-sulfones as molecular scaffold for the synthesis of pyrazolo[1,5-a]pyrimidine, 1,2,4-triazolo[1,5-a]pyrimidine and pyrimido[1,2-a]benzimidazole derivatives. The synthesized compounds were evaluated as Aurora-A kinase inhibitors and as anti-cancer tumor agents in order to explore new class of compounds that could be optimized for potent anticancer agents. In addition, docking study has been carried out to rationalize the biological activity.
2. Results and Discussion

2.1. Chemistry

Treatment of 1-aryl-2-(phenylsulfonyl)ethanones 1a–c with the appropriate heterocyclic amine and triethyl orthoformate in one pot, afforded the target fused heterocyclic ring systems. Thus, when compounds 1a–c were treated with 5-aminopyrazole derivatives 2a–g and triethyl orthoformate, in the presence of a catalytic amount of piperidine, they afforded the corresponding 1H-pyrazolo[1,5-a]pyrimidine derivatives 4a–l (Scheme 1). Moreover, the proposed reaction of the sulfone 1a with 5-aminopyrazole derivatives 2a–g and triethyl orthoformate in acetic anhydride and subsequent aromatization of so formed 1-aryl-3-ethoxy-2-(phenylsulfonyl)pyrimidine CH-2 in structure 3 with 5-aminopyrazole derivatives 2a–g and triethyl orthoformate (Scheme 1). A further evidence for the structures of the products 4a–l stems from their independent synthesis via condensation of 1a–c with triethyl orthoformate in acetic anhydride and subsequent condensation of so formed 1-aryl-3-ethoxy-2-(phenylsulfonyl)prop-2-ene-1-ones 3 with 5-aminopyrazole derivatives 2a–g which afforded products identical in all respects (m.p., mixed m.p. and IR) with those obtained from a three-component one step reaction of the sulfone 1 with the aminopyrazole derivatives 2a–g and triethyl orthoformate (Scheme 1). Moreover, the proposed structure of products 4a–l was further unequivocally confirmed by X-ray crystallography of compound 4b, taken as a typical example of prepared series (Fig. 1).

Similarly, when the sulfone derivatives 1a,b,d were treated with 2-aminobenzimidazole (6), under the same reaction conditions, they afforded, in each case, only one isolable product. The isolated products were identified as 2-aryl-3-(phenylsulfonyl)pyrimido[1,2-e]benzimidazole derivatives 5a–c (Scheme 2). The IR spectrum of the reaction products revealed, in each case, no bands due to amino or carbonyl functions. Moreover, the 1H NMR spectrum of compound 8a, taken as a typical example of the prepared series, revealed two singlet signals at δ 2.42 and 9.24 due to methyl and pyrimidine protons, respectively, in addition to an aromatic multiplet in region δ 7.15–7.95. The products 8a–c are assumed to be formed via the addition of the exocyclic amino group in 2-aminobenzimidazole to the activated double bond in the ethoxy-methylene derivative 3 to give the non-isolable acyclic intermediates 7a–c, which undergo intramolecular cyclization and subsequent aromatization via the loss of ethanol and water molecules, under the reaction conditions, to afford the final products 8a–c as depicted in Scheme 2.

The sulfone derivatives 1a,b reacted also with 3-amino-1,2,4-triazole (9) and afforded high yields of 7-aryl-6-(phenylsulfonyl)[1,2,4]triazolo[1,5-a]pyrimidine derivatives 10a,b (Scheme 3). The structures of the reaction products were assigned based on their elemental analyses and spectral data (cf. Experimental part).

2.2. Pharmacology

2.2.1. Anticancer activity

All newly synthesized compounds have been subjected to biochemical assays; i.e. Aurora-A inhibition activity, and cell-based assay using HCT116 colon tumor cell line in four deferent concentrations. Doxorubicin (Adriablastina®) was used as a reference drug in both assays. Aurora-A inhibition assay was summarized in Table 1, and in vitro cytotoxic was summarized in Table 2.

The parameter used in this study is half inhibition concentration (IC50), was calculated with regard to a saline control group and the potency was calculated with regard to the percentage of the change of the Doxorubicin and the tested compounds, as depicted in Table 1. In the present study, compounds that showed IC50 values more than 25 μg/ml were considered inactive.
2.3. Molecular modeling study

Molecular modeling study has been performed in order to rationalize the obtained biological results and get insight into the ligand–protein interactions. A molecular docking study was performed on all compounds, which were built with Sybyl 7.1 and minimized to 0.01 kcal/mol by the Powell method, using Gasteiger–Hueckel charges and the Tripos force fields. The energy-optimized compounds were docked into the active site of Aurora-A kinase, using GOLD software.

All active compounds showed relatively similar binding poses; therefore compound 4b was taken as a representative example of the series (Fig. 2A). These compounds suggested, by docking study, to be bound in an allosteric binding site 3–4 Å away from ADP binding site (Fig. 2A). In more details, the two phenyl rings at position 7 and that attached to sulfone moiety showed a strong edge-to-face π–π stacking with the phenyl ring of Thr219. This strong hydrophobic interaction is further supported by two hydrogen-bonds between sulfone moiety and Gln223 (Fig. 2B). These two interactions orient the rest of the molecule towards the magnesium ions deeply in the active pocket allowing the nitrogen atom at position 4 to create one more hydrogen-bond with the hydroxyl group of Thr213 (Fig. 2B). These two interactions orient the rest of the molecule towards the magnesium ions deeply in the active pocket allowing the nitrogen atom at position 4 to create one more hydrogen-bond with the hydroxyl group of Thr213 (Fig. 2B). In this particular example, there is no definite known role for phenyl ring at position-2, however, the observed higher activity of the corresponding 4-methoxy derivative 4c (Table 2) could be explained by the formation of an extra bond with magnesium ion (Fig. 2C). So far, the conducted computational model suggested that the methoxy-containing derivative 4c docked deeper than other corresponding compounds that lack the methoxy moiety. To test our computational model, the methoxy group was replaced with chlorine atom; therefore, compound 4d was synthesized. It was expected that the chlorine-containing analogue will reveal less activity than the corresponding methoxy group, since it has less ability for hydrogen-bond formation than oxygen. The aurora-A kinase assay came in accordance with the conducted computational model since it showed that compound 4d has one fifth inhibition activity of its methoxy analogue 4c (Table 1). On the other hand, it was assumed that the relatively similar cytotoxic activity of compounds 4c and 4d (Table 2) and the great difference in enzymatic inhibition assay (Table 1) could be due to other factors such as solubility and partition coefficient. This hypothesis is supported by the biological results of compound 4a which showed no significant cytotoxic activity in comparison with that of compounds 4b and 4c (Table 2). However, it revealed only 1–2 fold(s) IC50 values more than 4b and 4c, respectively, in the enzymatic inhibition assay (Table 1). This led us to the conclusion that certain degree of lipophilicity is required for the activity, and it was assumed that increasing the cLog P of compounds might lead to an increase in their cytotoxic properties. Therefore, Scheme 2 was adopted in which the phenyl group was fused with heterocyclic ring system and compounds 8a–8c were synthesized. Unfortunately, the enzymatic inhibition assay (Table 1) and cell cytotoxic assay (Table 2) of compounds 8a–8c showed no activity. The docking study suggests that the low activity of compounds 8a–8c may be due to the interference of the fused pyrimido[1,2-a]benzimidazole moiety with the methyl group of Thr217. The consequences of interference include docking of the compounds 3 Å away from original pose and inability for forming π–π stacking with Thr219. Next, as the fused phenyl group showed no biological activity, we decided to remove the phenyl group at position-2 to decrease the cLog P. So that, Scheme 3 was adopted and compounds 10a,b were synthesized. As expected, decreasing the lipophilicity led to a decrease in the cytotoxic properties of the prepared compounds (Table 2).

The docking study suggests that substitution of phenyl ring at position-8 (compounds 4g–l) could interfere with the C-backbone of Pro259, Gln260 and Asn261 amino acid residues. However, there is no definite explanation for the moderate activity of the chlorinated compound 10b in comparison with 4g–l. It was assumed that, the lacking of substitution at position-8 may provide more flexibility in the active pocket. The moderate cytotoxic effect of compound 10b may be due to the interference with other proteins rather than the Aurora-A kinase itself. This explanation is supported by the high IC50 value of compound 10b in Aurora-A kinase inhibition assay (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>Compound</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>0.040</td>
<td>4i</td>
<td>NA</td>
</tr>
<tr>
<td>4a</td>
<td>0.101</td>
<td>4j</td>
<td>NA</td>
</tr>
<tr>
<td>4b</td>
<td>0.051</td>
<td>4k</td>
<td>NA</td>
</tr>
<tr>
<td>4c</td>
<td>0.025</td>
<td>4l</td>
<td>NA</td>
</tr>
<tr>
<td>4d</td>
<td>0.120</td>
<td>8a</td>
<td>NA</td>
</tr>
<tr>
<td>4e</td>
<td>NA</td>
<td>8b</td>
<td>NA</td>
</tr>
<tr>
<td>4f</td>
<td>0.039</td>
<td>8c</td>
<td>NA</td>
</tr>
<tr>
<td>4g</td>
<td>NA</td>
<td>10a</td>
<td>0.104</td>
</tr>
<tr>
<td>4h</td>
<td>NA</td>
<td>10b</td>
<td>9.238</td>
</tr>
</tbody>
</table>

NA: not active.

### Table 2

*In vitro* cytotoxic activity of the newly synthesized compounds and doxorubicin against HCT116 colon tumor cell line.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fraction surviving</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg/ml)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.523</td>
<td>0.439</td>
</tr>
<tr>
<td>4a</td>
<td>0.774</td>
<td>0.698</td>
</tr>
<tr>
<td>4b</td>
<td>0.683</td>
<td>0.455</td>
</tr>
<tr>
<td>4c</td>
<td>0.516</td>
<td>0.436</td>
</tr>
<tr>
<td>4d</td>
<td>0.560</td>
<td>0.508</td>
</tr>
<tr>
<td>4e</td>
<td>0.529</td>
<td>0.373</td>
</tr>
<tr>
<td>4f</td>
<td>0.629</td>
<td>0.420</td>
</tr>
<tr>
<td>4g</td>
<td>0.987</td>
<td>0.953</td>
</tr>
<tr>
<td>4h</td>
<td>0.824</td>
<td>0.794</td>
</tr>
<tr>
<td>4i</td>
<td>1.00</td>
<td>0.745</td>
</tr>
<tr>
<td>4j</td>
<td>1.00</td>
<td>0.701</td>
</tr>
<tr>
<td>4k</td>
<td>1.00</td>
<td>0.665</td>
</tr>
<tr>
<td>4l</td>
<td>1.00</td>
<td>0.764</td>
</tr>
<tr>
<td>8a</td>
<td>0.984</td>
<td>0.885</td>
</tr>
<tr>
<td>8b</td>
<td>0.950</td>
<td>0.905</td>
</tr>
<tr>
<td>8c</td>
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<td>0.748</td>
</tr>
<tr>
<td>10a</td>
<td>0.689</td>
<td>0.302</td>
</tr>
<tr>
<td>10b</td>
<td>0.786</td>
<td>0.377</td>
</tr>
</tbody>
</table>
3. Experimental

3.1. Chemistry

3.1.1. General
All melting points were measured on a Gallenkamp melting point apparatus (Weiss-Gallenkamp, London, UK). The infrared spectra were recorded in potassium bromide disks on a pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometers (Pye Unicam Ltd, Cambridge, England and Shimadzu, Tokyo, Japan, respectively). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian, Palo Alto, CA, USA). \(^1\)H spectra were run at 300 MHz and \(^1\)C spectra were run at 75.46 MHz in deuterated chloroform (CDCl\(_3\)) or dimethyl sulphoxide (DMSO-d\(_6\)). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu) at 70 eV. Elemental analyses were carried out at the Micro-analytical Center of Cairo University, Giza, Egypt.

X-ray crystallography was carried out on Kappa CCD Enraf Nonius FR 590 diffractometer, National Research Center, Dokki, Cairo, Egypt.

The sulfone derivatives 1a–d [27], 1-aryl-3-ethoxy-2-(phenylsulfonyl)prop-2-ene-1-one 3 [28], pyrazolo[1,5-a]pyrimidines 4i–l [19], and pyrimido[1,2-a]benzimidazoles 8c [19], were prepared according to the reported literature.

3.1.2. Pyrazolo[1,5-a]pyrimidines 4a–e, pyrimido[1,2-a]benzimidazole 8a–c and triazolo[1,5-a]pyrimidine derivatives 10a,b

3.1.2.1. General procedure

3.1.2.1.1. Method A. A solution of 1-aryl-2-(phenylsulfonyl)ethanone (1a) (10 mmol) and an equivalent molar ratio of the appropriate heterocyclic amine 2, 6 or 9 in triethyl orthoformate (20 mL), in the presence of piperidine (0.3 mL), was heated under reflux for 4 h. The excess solvent was removed by distillation under reduced pressure and the residue was left to cool. The precipitated solid product was collected by filtration, washed with ethanol, dried and finally recrystallized from DMF/EtOH to afford the corresponding pyrazolo[1,5-a]pyrimidines 4a–e, pyrimido[1,2-a]benzimidazoles 8a–c and triazolo[1,5-a]pyrimidine derivatives 10a,b, respectively.

3.1.2.1.2. Method B. To a mixture of 1-aryl-3-ethoxy-2-(phenylsulfonyl)prop-2-en-1-one 3 [28] (10 mmol) and appropriate aminopyrazole derivative 2 (10 mmol) in absolute EtOH (25 mL) were added few drops of piperidine and the reaction mixture was refluxed for 1 h, then left to cool. The formed solid product was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford products identical in all respects (mp, mixed mp and spectra) with those obtained by Method A above.

The physical and spectral data of the newly synthesized compounds 4a–h, 8a,b and 10a,b are listed below.

3.1.2.2. 2-Methyl-7-phenyl-6-(phenylsulfonyl)pyrazolo[1,5-a]pyrimidine (4a). Yield (82%); m.p. > 300 °C; IR (KBr) \(\nu_{\text{max}}/\textrm{cm}^{-1}\): 1590 (C=N), 1147, 1318 (SO2); \(^1\)H NMR (DMSO-d\(_6\)): \(\delta\) 2.31 (s, 3H, CH3), 6.76 (s, 1H, pyrazole-3-CH), 7.13–7.54 (m, 10H, ArH), 9.12 (s, 1H, pyrimidine-5-CH); \(^1\)C NMR (DMSO-d\(_6\)): \(\delta\) 14.48, 97.64, 120.78, 127.11, 127.37, 127.72, 129.02, 129.36, 129.56, 130.21, 140.48, 146.95, 148.33, 149.60, 158.18; MS (m/z): 349 (M\(^+\)). Anal. Calcd for C\(_{19}\)H\(_{15}\)N\(_3\)O\(_2\)S (349.41): C, 65.31; H, 4.33; N, 12.03; S, 9.18%. Found: C, 65.40; H, 4.36; N, 12.00; S, 9.11%.

3.1.2.3. 2,7-Diphenyl-6-(phenylsulfonyl)pyrazolo[1,5-a]pyrimidine (4b). Yield, (75%); m.p. 252 °C; IR (KBr) \(\nu_{\text{max}}/\textrm{cm}^{-1}\): 1582 (C=N), 1154, 1308 (SO2); \(^1\)H NMR (DMSO-d\(_6\)): \(\delta\) 7.31 (s, 1H, pyrazole-3-CH), 7.31–7.54 (m, 10H, ArH), 9.12 (s, 1H, pyrimidine-5-CH); \(^1\)C NMR (DMSO-d\(_6\)): \(\delta\) 14.48, 97.64, 120.78, 127.11, 127.72, 129.02, 129.36, 129.56, 130.21, 140.48, 146.95, 148.33, 149.60, 158.18; MS (m/z): 349 (M\(^+\)). Anal. Calcd for C\(_{22}\)H\(_{18}\)N\(_3\)O\(_2\)S (349.41): C, 65.31; H, 4.33; N, 12.03; S, 9.18%. Found: C, 65.40; H, 4.36; N, 12.00; S, 9.11%.

Fig. 2. (A and B) Binding mode of proposed 1st ranked pose for compound 4b into the catalytic site of Aurora-A kinase enzyme; (C) Binding mode of proposed 1st ranked pose for compound 4c into the catalytic site of Aurora-A kinase enzyme. The important amino acids were represented as green sticks. Mg ions were represented as gray spheres.
7.22—7.77 (m, 15H, ArHs), 9.17 (s, 1H, pyrimidine-5-CH); 13C NMR (DMSO-d6): δ 94.98, 120.51, 125.16, 126.29, 127.04, 127.10, 127.56, 128.62, 128.91, 129.47, 130.27, 133.16, 134.46, 140.39, 147.21, 148.57, 150.20, 158.03; MS (m/z): 411 (M+); Anal. Calcld for C24H16N4O2S (445.92): C, 68.10; H, 4.30; N, 9.49; S, 7.24%. Found: C, 68.65; H, 3.96; N, 10.83; S, 8.26%.

3.1.2.11. 4-(4-Chlorophenyl)-3-(phenylsulfonyl)pyrimido[1,5-a]imidazole (8b). Yield (80%); mp > 300 °C; IR (KBr) vmax/cm−1: 1604 (C–N), 1159, 1316 (SO2); 1H NMR (DMSO-d6): δ 114.06, 114.88, 120.62, 122.97, 127.25, 127.30, 127.57, 127.81, 127.89, 129.27, 133.47, 136.58, 139.83, 141.27, 144.79, 161.48; MS (m/z): 385 (M+). Anal. Calcld for C22H14N4O2S (385.44): C, 68.55; H, 3.92; N, 10.90; S, 8.32%. Found: C, 68.65; H, 3.96; N, 10.83; S, 8.26%.

3.1.2.12. 7-(4-Chlorophenyl)-6-(phenylsulfonyl)-1H-[1,2,4]triazolo[1,5-a]pyrimido[1,5-a]imidazole (10a). Yield (83%); mp > 300 °C; IR (KBr) vmax/cm−1: 1615 (C=–N), 1151, 1312 (SO2); 1H NMR (DMSO-d6): δ 7.36–7.76 (m, 10H, ArHs), 10.69 (s, 1H, pyrimidine-5-CH); 13C NMR (DMSO-d6): δ 124.28, 126.37, 126.77, 124.77, 127.71, 129.01, 130.81, 133.91, 139.92, 150.52, 153.37, 157.56, 157.74; MS (m/z): 336 (M+). Anal. Calcld for C17H13N4O2S (336.37): C, 60.70; H, 3.60; N, 16.66; S, 9.53%. Found: C, 60.79; H, 3.55; N, 16.62; S, 9.53%.

3.2. Pharmacology

3.2.1. In vitro anti-tumor screening

All newly synthesized compounds were subjected to the in vitro disease-oriented primary anti-tumor screening against human HCT116 colon tumor cell line. The human tumor cell line of the cancer screening panel was grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM l-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 mL at plating densities 10,000 cells/well. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates were fixed in situ with TCA, to represent a measurement of the cell population at the time of compound addition. Experimental compounds as well as Doxorubicin were solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg/mL gentamicin. Additional four 10-fold or 1/2 log serial dilutions were made to provide a total of four drug concentrations (1, 2.5, 5 and 10 μg/mL) plus control. Aliquots of 100 mL of these different drug dilutions were added to the appropriate microtiter wells already containing 100 mL of medium, resulting in the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO2, 95% air, and 100% relative humidity.
For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 mL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 mL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was recovered with Tris–EDTA buffer. Color intensity was measured in ELISA reader.

The relation between surviving fraction and drug concentration is plotted to get the survival curve of HCT116 tumor cell line. The IC50, was calculated for each trial [29–31].

3.2.2. Aurora assays

As prescribed by Fancelli et al. [32], the Aurora proteins were produced in insect cells as a GST-fusion protein and purified using GST affinity chromatography and gel filtration. The biochemical activity of compounds was determined by incubation with Aurora kinase and substrate, followed by quantitation of the phosphorylated product. Compounds were 3-fold serially diluted from 10 μM to 0.0005 μM and then incubated for 60 min at room temperature in the presence of ATP/P [33]; γ-ATP mix (10 μM), CHOKTide 4× (8 μM) for Aurora-A (2.5 mM); ATP/P [33] γ-ATP mix (20 μM), CHOKTide 4× (8 μM) in a final volume of 30 μL of buffer (HEPES pH 7.5 50 mM, MgCl2 10 mM, DTT 1 mM; NaVO3, 3 μM + 0.2 mg/mL BSA); using 96 U bottom plates. After incubation, the reaction was stopped by the addition of 100 μL of PBS + 32 mM EDTA + 0.1% Triton X-100 + 500 μM ATP, containing 1 mg streptavidin-coated SPA beads (biontin capacity 130 pmol/mg). After 20 min incubation for substrate capture, 100 μL of the reaction mixture was transferred into Optiplates (PerkinElmer) 96-well plates containing 100 μL of 5 M CsCl, left to stand for 4 h to allow stratification of beads to the top of the plate, and counted using TopCount (Packard) to measure substrate-incorporated phosphate.

3.3. Molecular modeling

All newly synthesized compounds were built in Sybyl 7.1 software and minimized to 0.01 kcal/mol by the Powell method, using Gasteiger–Huckel charges and the Tripos force field. To save calculation time, solvents were not taken into account, and instead the dielectric constant was set to a value of 4 to mimic the aqueous environment. The minimized molecules underwent 10 rounds of simulated annealing to search for the optimized conformation. During the simulation process, the starting conformation in each round was heated to 700 K within 1000 fs and then cooled to 200 K in the same period. At the each temperature level, conformations were collected and recorded. The conformers located at the starting point at the each round of simulation were selected for the further energy refinement using the same parameter set as the ones in molecular construction. The minimized conformer with the lowest energy was selected as the optimized conformation of the molecule which will be docked into the Aurora-A kinase.

The crystallographic coordinates of Aurora-A kinase were obtained from the Protein Databank (PDB ID: 2MQ4). The energy-optimized compounds were docked into the Aurora-A kinase. In this work, the active site radius was taken as 8 Å from the ADP binding site. The parameters were set as the default value that GOLD suggested. The center of the binding domain was defined as original point (0, 0, 0) of the X, Y, Z axes that were used as the referenced point of the GOLD docking program.

4. X-ray crystallography

A single crystal of compound 4b was obtained by slow evaporation from a mixture of ethanol: DMF (2:1). The crystal structure was solved and refined using maxus (nonius, Defilt and MacScience, Japan) [33] Mo-Kz radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection.

The chemical formula and ring labeling system are shown in Fig. 1.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 687842. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: 144 (0)-1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].