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Synthesis and molecular modelling studies of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines as multitarget Ser/Thr kinases inhibitors

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ABSTRACT

This paper reports the design and synthesis of a novel series of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines *via* microwave-assisted multi-step synthesis. A common precursor of the whole series, 3-amino-5-bromothieno[2,3-*b*]pyridine-2-carbonitrile, was rapidly synthesized in one step from commercially-available 5-bromo-2-chloronicotinonitrile. Formylation with DMF-DMA led to (*E*)-*N*′-(5-bromo-2-cyanothieno[2,3-*b*]pyridin-3-yl)-*N*,*N*-dimethylformimidamide (**4**) which was conveniently functionalized at position 8 by palladium-catalyzed Suzuki-Miyaura cross-coupling to introduce a heteroaromatic ring. High-temperature formamide-mediated cyclization of the cyanoamidine intermediate gave seventeen 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines. The inhibitory potency of the final products was evaluated against five protein kinases (CDK5/p25, CK1δ/ε, GSK3α/β, DYRK1A and CLK1) and revealed that 8-(2,4-dichlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine **1g** specifically inhibits CK1δ/ε and CLK1 (220 and 88 nM, respectively) while its 7-(2,4-dichlorophenyl) pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine isomer **10** showed no activity on the panel of tested kinases. Molecular modelling of **10** and **1g** in the ATP binding sites of CK1δ/ε and CLK1 showed that functionalization at position 7 of pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines is likely to induce a steric clash on the CK1δ/ε P-loop and thus a complete loss of inhibitory activity.

1. Introduction

Protein kinases catalyze protein phosphorylation, a key cellular regulatory mechanism which is frequently deregulated in human diseases. All major diseases, including cancer, neurodegenerative disorders and cardiovascular diseases have been closely associated with kinases' deregulations [1–3]. Consequently, protein kinases and their potential inhibitors represent valuable targets for the pharmaceutical industry in its search for new therapeutic agents. Most kinases act on both serine and threonine, others act on tyrosine, and a number (dual-specificity kinases) act on all three amino acids. Our research groups are mainly invested in the synthesis of C,N,S- or C,N,O-containing heterocyclic precursors of bioactive molecules able to modulate the activity of kinases in signal transduction [4–8] and especially Ser/Thr kinases (CDK5, GSK3, CLK1 and CK1) and dual-specificity kinases (DYRK1A) [4–9]. These eukaryotic kinases belong to a larger family known as the CMGC group of serine/threonine kinases. Since these five kinases are all involved to some extent in Alzheimer's disease (AD), a multi-kinase inhibitor able to target more than one of them may provide a highly pertinent therapeutic strategy for patients suffering of this neurodegenerative disease. Although recent works seem to demonstrate the interest of multi-target-directed ligands (MTDLs) [10], it still remains unknown whether single or multi-selective inhibitors are most suited for Alzheimer's disease drug development. This uncertainty mainly lies in the fact that it still remains

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unresolved to what extent each of these five kinases plays a more prominent role in Alzheimer's disease than the others and, thus, which one should preferably be targeted.

In the course of our work, *N*-arylbenzothieno[3,2-*d*]pyrimidin-4-amines and their pyrido and pyrazino analogues were described [6–8]. These original heteroaromatics provide new means to target and inhibit some of the above-mentioned kinases in the nanomolar range. It was found that *N*-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine series (see **I** in Scheme 1) turned out to be particularly promising for the development of new pharmacological inhibitors of CK1, CLK1 and DYRK1A kinases. Among these compounds, **II** (Scheme 1) was identified as the most active product with low IC_{50} values for CK1 (31 nM) and CLK1 (680 nM). This compound also displayed a micromolar value for DYRK1A (2.8 μM) [6b]. Pursuing our research on the design of original kinase inhibitors, we wished to synthesize and investigate the biological potential of a new series (series 1) of C8-substituted pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (Scheme 1).

The choice to introduce heteroaryl substituents in position 8 of the tricyclic structure was guided by our previous endeavour to synthesize C7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine derivatives [7] and some of their pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine analogues (**III** in Scheme 1), most of which showed moderate affinities for the five targeted Ser/Thr kinases (CDK5, CK1, CLK1, DYRK1A and GSK3).

This article describes the development of a simple and reliable synthetic protocol that allowed preparation of a library of new C8-substituted pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidines (series **1**) for which interesting kinase inhibitory activities were observed. The main part of the chemistry described in this paper was carried out under microwave irradiation in a combinatorial chemistry approach. Since we thought it would be more appropriate to test all brain isoforms at once as they are expressed in the brain, the target tissue, the evaluation of kinase inhibition was performed on a panel of five Ser/Thr kinases and their isoforms. Docking studies were achieved in hope to understand and confirm the interactions of the best products in the ATP binding sites.

2. Chemistry

The synthetic route to accomplish the synthesis of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine derivatives (**1**) is depicted in Scheme 2. This multi-step synthetic pathway was inspired by our previous works on the efficacy of formamide degradation. Microwave-assisted degradation of formamide is well known for generating ammonia *in situ* and cyclizing cyanoamidines into pyrimidin-4-amine rings via a nitrogen atom introduction, from anthranilonitrile, or its benzofuro or benzothieno analogue [11].

The cyanoamidine (**4**) could be synthesized in two steps from 5-bromo-2-chloronicotinonitrile (**2**). This intermediate could be converted into the expected tricyclic pyrimidin-4-amine after a Suzuki-Miyaura cross-coupling reaction with various heteroarylboronic acids in order to insert the aryl ring in position 8. Final cyclization into pyrimidin-4-amine would be achieved by nucleophilic attack of ammonia generated *via* thermal degradation of formamide. The order of this synthetic sequence was defined after the preliminary exploration of a similar synthesis [12a]. It was discovered that the most effective route to access such derivatives implied the use of formamide in the last step of the sequence.

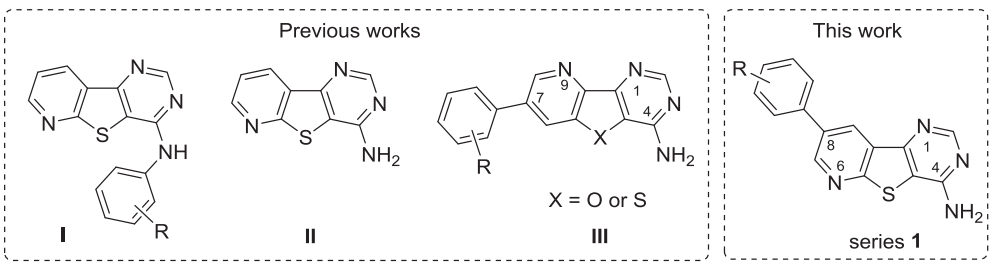
Experimentally, 5-bromo-2-chloronicotinonitrile (**2**) was treated with freshly prepared 3-mercaptopropionitrile [12] and aqueous potassium hydroxide, in dimethylformamide (DMF) at 0 °C. After 1 h of stirring, bromoacetonitrile was added to the cold reaction mixture to give the expected 3-amino-5-bromothieno[2,3-*b*]pyridine-2-carbonitrile (**3**) in high yield (91%). Then, (*E*)-*N*′-(5-bromo-2-cyanothieno[2,3-*b*]pyridin-3-yl)-*N*,*N*-dimethyl formimidamide intermediate (**4**) was prepared in quantitative yield by reaction of cyanoenamine **3** with *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) after 30 min of microwave heating (90 °C) (Scheme 3).

Introduction of phenyl substituents prior to the pyrimidin-4-amine ring formation was accomplished by Suzuki cross-coupling of various heteroarylboronic acids with intermediate **4** in usual conditions of temperature and time to give *(E)*-*N*′-(5-aryl-2-cyano) thieno[2,3-*b*]pyridin-3-yl)-*N*,*N*-dimethylformimidamide series (**5a**–**q**) in very good yields (Table 1). Strong microwave-assisted heating (185 °C) of these highly reactive intermediates with formamide allowed synthesis of novel 8-(het)arylpyrido[3′,2′:4,5] thieno[3,2-*d*]pyrimidin-4-amines (**1a**–**q**) in good overall yields (55–100%) [13].

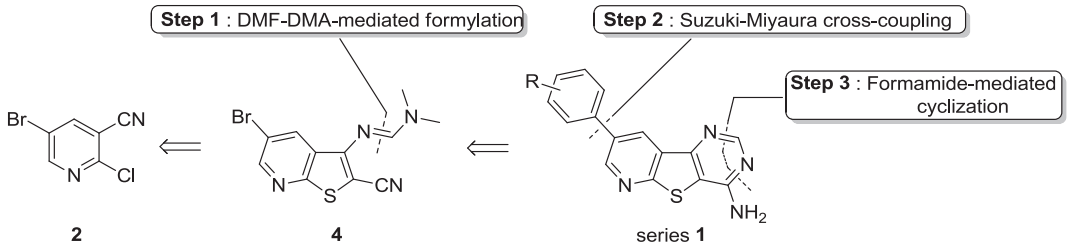
In order to confirm the real impact of the functionalization in position 8 of series **1**, a short study was undertaken. We wished to synthesize an isomeric derivative of the best inhibitor of the previous series. We focused our attention on the preparation of a regioisomer of pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidine **1g**. The target molecule (see **10** in Scheme 4) would also bear a 2,4-dichlorophenyl substituent at position 7 of the ring instead of position 8. While the pyrimidinic moiety would remain unaffected, a change in the spatial constraints of the pyridinic ring may induce important alterations of the biological activity, hence proving the importance of the substitution in position 7 or 8.

After preliminary investigations, the chosen synthetic route was adapted from previous work on regioselective cross-coupling reactions of boronic acids with dihaloheterocycles [14,15]. Commercial 2,6-dichloropyridine-3-carbonitrile (**6**) was thus chosen as the starting material to access isomer **10**. Microwave-assisted Suzuki-Miyaura cross-coupling of **6** with 2,4-dichlorophenylboronic acid, sodium carbonate, and $Pd(PPh_3)_4$, in a dioxane/water (3:1, v/v) at 100 °C (400 W) yielded 2-chloro-6-(2,4-dichlorophenyl)nicotinonitrile (**7**) in excellent 87% yield after 2 h of reaction (Table 1).

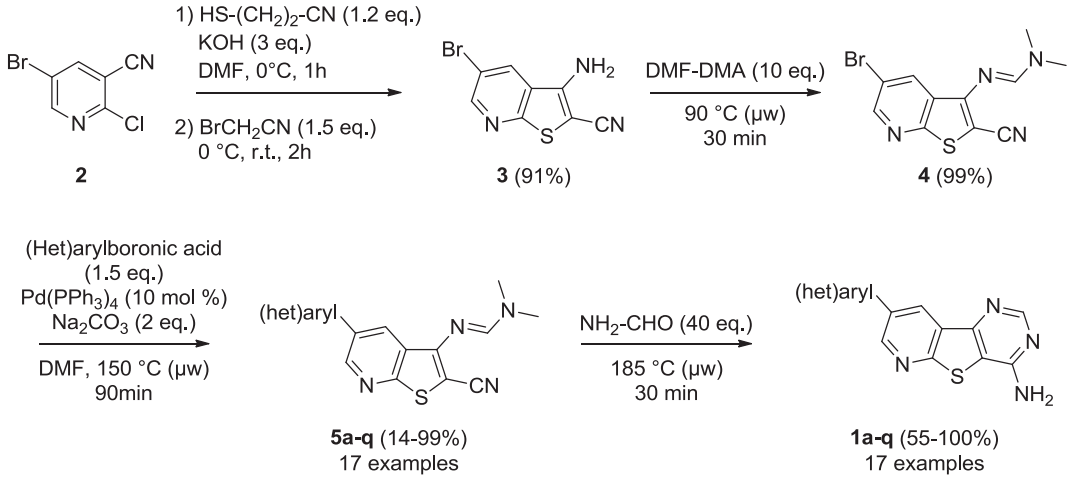
The rest of the synthesis was achieved according to the route described in Scheme 3. Accordingly, 6-substituted 2-



Scheme 1. Structures of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine targets (series **1**), their pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine and *N*-phenyl analogues (**I** and **II**) [6], and furo- or thieno[3,2-*d*]pyrimidin-4-amine isomers (**III**) [8].



Scheme 2. Synthetic route envisioned for access to the target 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (series **1**).



Scheme 3. Synthesis of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (**1a**–**q**); for detailed yields see Table 1.

chloronicotinonitrile **7** was successively treated with mercaptopropionitrile and bromoacetonitrile in aqueous potassium hydroxide and DMF at 0 °C to give the expected 3-amino-6-(2,4-dichlorophenyl)thieno[2,3-*b*]pyridine-2-carbonitrile (**8**) in modest 28% yield.

Then, (*E*)-*N*′-[2-cyano-6-(2,4-dichlorophenyl)thieno[2,3-*b*]pyridin-3-yl]-*N*,*N*-dimethyl formimidamide intermediate (**9**) was prepared in 73% yield by reaction of cyanoenamine **8** with DMF-DMA after 30 min of microwave heating at 90 °C. Strong microwave heating (185 °C) of the highly reactive intermediate **9** in the presence of formamide allowed convenient synthesis (87% yield) of the expected 7-(2,4-dichlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine (**10**), the regioisomeric product of **1g**.

At this stage of the study, the synthesis of seventeen 8-substituted pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines was performed with success. Suzuki cross-coupling reactions were used to functionalize pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines with n heteroaryl ring in position 8. An isomer **10** of one of the most active compound **1g** was also prepared *via* a preliminary and regioselective cross-coupling reaction.

Note that microwave heating was mainly performed at atmospheric pressure in a controlled multimode cavity with a microwave power delivery system ranging from 0 to 1200 W. Concerning the technical aspect, the choice of a reactor able to work at atmospheric pressure was guided by our previous experience in microwave-assisted heterocyclic syntheses, especially in the chemistry of quinazolines [16]. Open vessel microwave experiments have some advantages, such as the possibility of easier scale-up and the possibility to use common laboratory glassware. Our choice was also guided by a recent work describing the tendency of pressure to accumulate when a product as DMF-DMA was heated into pressurized vials, especially under microwaves [11]. In most cases, a 600–800 W irradiation was enough to efficiently reach the programmed temperature. This parameter was mainly monitored *via* a contactless-infrared pyrometer which was calibrated in control experiments with a fibre-optic contact thermometer.

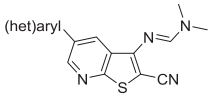
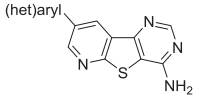
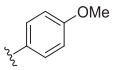
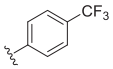
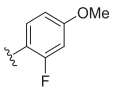
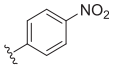
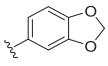
3. Biological activities

The final products (**1a**–**q**) and **10** were tested on five different *in vitro* kinase assays (CDK5/p25 (cyclin-dependent kinase), CK1δ/ε (casein kinase 1), GSK3α/β (glycogen synthase kinase 3), DYRK1A (dual-specificity tyrosine phosphorylation regulated kinase) and CLK1 (cdc2-like kinase 1) to evaluate their inhibition potency [17–20]. All compounds were first tested at a final concentration of 10 μM. Compounds showing less than 50% inhibition were considered as inactive (IC_{50} > 10 μM). Compounds displaying more than 50% inhibition at 10 μM were next tested over a wide range of concentrations (usually 0.01–10 μM), and IC_{50} values were determined from the dose–response curves (Sigma-Plot). Harmine (entry 20 in Table 2) is a β-carboline alkaloid known to be a potent inhibitor of DYRK1A [21]. It was also tested as positive control and its IC_{50} value was compared to those obtained for the compounds under study. Results of the most active compound (see **II** in Scheme 1) in the preceding study [6b] were obtained in the same assays and were added to Table 2 (entry 19).

Results given in Table 2 demonstrate that none of the tricyclic derivatives prepared in this work showed affinity against CDK5/p25 and GSK3. On a general aspect, the biological activity of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (**1a**–**q**) and one of their isomer, **10**, was oriented towards three kinases of the initial panel: CK1, CLK1 and DYRK1A.

Undoubtedly, the most active molecules prepared in this study were series **1i**–**l** and compounds **1g** and **1p**. These compounds

Table 1
Synthesis of *N*,*N*-dimethylformimidamides **5a**–**q** and final thieno[3,2-*d*]pyrimidines **1a**–**q**.

(Het)Aryl				
	Compound N°	Yield (%)[a,b,c]	Product N°	Yield (%)[b,c,d]
	**5a**	99	**1a**	87
	**5b**	99	**1b**	93
	**5c**	79	**1c**	85
	**5d**	94	**1d**	78
	**5e**	98	**1e**	92
	**5f**	55	**1f**	99
	**5g**	90	**1g**	73
	**5h**	64	**1h**	64
	**5i**	70	**1i**	66
	**5j**	67	**1j**	66
	**5k**	88	**1k**	79
	**5l**	99	**1l**	61
	**5m**	14	**1m**	65
	**5n**	22	**1n**	55
	**5o**	64	**1o**	93
	**5p**	79	**1p**	87
	**5q**	54	**1q**	67

[a] Reactions were performed for 90 min at 150 °C and at atmospheric pressure under microwave irradiation (800 W) on a 1.0 mmol scale from **4** with 1.5 equiv of appropriate boronic acid.
[b] Yield of isolated product.
[c] Microwave reactor: RotoSYNTH™ from Milestone S.r.l, Italy.
[d] Reactions were performed for 30 min at 185 °C and at atmospheric pressure under microwaves (200 W) on a 0.2 mmol scale from **5a**–**q** with 40 equiv of formamide.

showed submicromolar activities against CK1 (0.19μM < IC_{50} < 0.50 μM) and CLK1 (0.071μM < IC_{50} < 0.19 μM) with a slight preference for the second one. The DYRK1A IC_{50} values obtained for all these compounds are situated mainly in the micromolar range (1.1μM < IC_{50} < 11 μM) except for compounds **1g** (entry 7) and **1k** (entry 11) for which submicromolar IC_{50} values (0.53 and 0.33 μM, respectively) were observed.

Comparison of the results obtained for compounds **1a**–**f** (entries 1–6), **1m**–**o** (entries 13–15), and **1q** (entry 17) with those obtained for **1g** (entry 7) and **1i**–**l** (entries 9–12), demonstrates that the presence of two substituting groups on the phenyl ring in position 8 seems to have a beneficial effect on the inhibitory activity. Without being able to establish a general rule, the presence of substituents in positions 2 and 4 of the aromatic ring in position 8 of the main core has a rather beneficial effect compared to para-substituted derivatives.

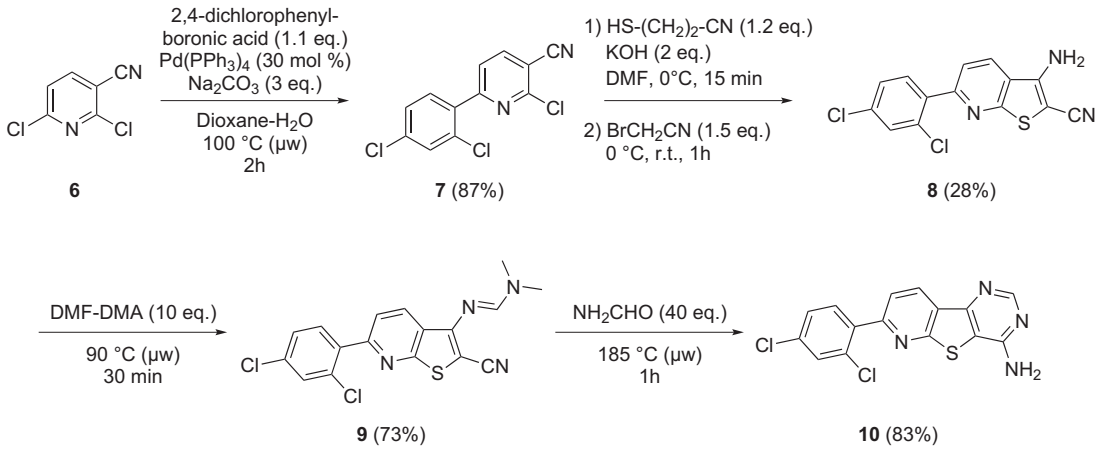
Biological results obtained for compound **1p**, (entry 16: 0.19 μM and 0.18 μM for CK1 and CLK1, respectively) are remarkable. **1p** is the only derivative with a thiophenic ring in place of the phenyl group that can inhibit the activity of CK1 and CLK1, indicating that other thiophene-substituted derivatives could be bioisosteres of their phenyl-substituted analogues of this study. Values given in Table 2 (entry 16) are similar to those obtained for the 2,4-disubstituted phenyl leaders (**1g** and series **1i**–**1l**). Compared to the unsubstituted phenyl precursor **1a** (entry 1), the thiophenic derivative **1p** may be considered as slightly more active although their IC_{50} values against CLK1 (0.18 and 0.19 μM) were similar.

Biological data for the isomeric derivative **10** (entry 18) and those obtained for **1g** (entry 7) prove that changing the 2,4-dichlorophenyl substituent from position 8 (series **1**) to position 7 (compound **10**) dramatically decreased the affinity of the most active inhibitor of this series against kinase CK1. Product **10** completely lost its affinity against CK1 and showed a quite selective IC_{50} value against CLK1 (0.1 μM), whilst the affinity measured for DYRK1A (IC_{50} = 6.1 μM) was found modest and not really significant.

All these results demonstrate that it is difficult to define any role for the various substituents of the aryl group located at position 8. Noteworthy, the presence of two halogen atoms on the aromatic rings (**1g** and **1i**–**l**, Table 2) resulted in submicromolar affinity for CK1 and also for CLK1 in a large number of the active products, although the observed affinities were lower for DYRK1A. On the contrary, the presence of one substituent on the *N*-aryl moiety decreased the inhibitory activity on all the other tested kinases.

CK1 is a Ser/Thr protein kinase family consisting of multiple isoforms (α, β, γ1–3, δ and ε) which are highly homologous within their kinase domains. Recently, several studies have highlighted the overactivity of some CK1 isoforms in neurodegenerative diseases, especially in tauopathies such as Alzheimer disease (AD) [22]. All these results demonstrated that the two CK1δ and CK1ε isoforms seem to be the most interesting to target and to modulate as a therapeutic strategy [23]. It was also highlighted that CK1δ/ε isoforms are also key regulators of the Wnt signalling pathway and consequently, may have a regulation role in cell survival and cancer progression. As the involvement of CK1 in cell regulation and neurodegenerative diseases pathogenesis has recently been identified, there has been relatively little work regarding inhibitors of this family of protein kinases [24].

CLK1 is one of the four isoforms (CLK1-4) of the cdc2-like kinase family. In humans, the highest levels of CLK1 expression were found in the brain. It was described that inhibitors of CLK1 could prove to be useful agents in disease phenotypes characterized by abnormal mRNA splicing. In this sense CLK inhibitors may alter the splicing of microtubule-associated protein tau implicated in Alzheimer's disease and Parkinson's disease [25].



6 **7** (87%) **8** (28%) **9** (73%) **10** (83%)

Scheme 4. Multistep synthesis of 7-(2,4-dichlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amine (**10**).

The third kinase targeted by the compounds described in this study was DYRK1A. Evidence for the role of overexpressed DYRK1A in various neurodegenerative diseases and Down syndrome is now well established and it has become an attractive drug target for numerous research groups [26].

Recently, low benefits of drugs which focused their activity against a single target encouraged new studies in the direction of multi-targeting strategies. Therefore, developing a drug which has micromolar or submicromolar affinities on a panel of two or three kinases seems to be of great interest. Such inhibitors should be able to regulate the targeted kinases without affecting the basic activity in cells. In this sense the new series described in this paper may lead to the discovery of valuable multi-kinases inhibitors.

4. Molecular modelling

Molecular modelling studies were performed to understand the nature of interactions governing the binding mode of these molecules in the active sites of CK1 and CLK1. To this end, compounds **1g** and **10** were docked into crystal structures of CLK1 in complex with debromohymenialdisine at 1.70 Å resolution (pdb code: 1Z57) [27] and/or CK1δ in complex with PF4800567 at 2.07 Å resolution (pdb code: 4HNF) [28]. Interestingly, CK1 inhibitor PF4800567 (Fig. 1) also displays a pyrimidin-4-amine group that makes classical H-bond interactions with the hinge region of the kinase. These interactions are similar to those established by the adenine moiety of ATP in the active site, with the exception of the *meta*-chlorophenyl moiety of PF4800567 which binds in the hydrophobic pocket adjacent to the CK1δ gatekeeper residue Met82 that could likely account for the high selectivity over other kinases [29,30]. Thereby, even if different binding modes in the ATP site could be involved with our molecules, we applied distance constraint between the common aminopyrimidine core and the hinge domains formed either by the CLK1 residues Glu242 and Leu244 or by the CK1δ residues Glu83 and Leu85. In this way, a similar binding pose was found with the 2,4-dichlorophenyl group (**1g**) oriented towards the phosphate binding loop (P-loop) (Fig. 2), a highly flexible region of kinases [31]. On CLK1, this group can establish additional π-π interactions with Phe172, a conserved aromatic residue that may greatly *contribute to the biological activity* compared to non-substituted compound **II** (Fig. 2a), while on CK1, the corresponding amino acid (Phe20) is more distant from the active site and may not have any impact on the binding affinity (Fig. 2b). Also, the loss of activity observed with analogue **10** on CK1 may result from a steric clash between the P-loop and the displaced 7-aryl group, such a situation would seem to be partially avoided with CLK1 (Fig. 2c).

5. Conclusion

A chemical library of seventeen novel 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines **1a**–**q** was rapidly achieved using microwave-assisted synthesis. Good control of the reaction parameters allowed efficient heating of the reaction mixture, resulting in short reaction times and high yields. The inhibitory potency of the final products against five kinases involved in AD was evaluated. Also, a 7-substituted isomer (**10**) of one of the most active compound (**1g**) was successfully prepared and evaluated biologically. Our study demonstrates that several molecules described in this paper are particularly promising for the development of new multi-target inhibitors of CK1 and CLK1 kinases. Although the affinity of these compounds for DYRK1A is not negligible, it still needs optimization to provide a synergetic effect to these molecules. The most active compounds **1g** and **1p** showed submicromolar inhibition and a relative selectivity for CK1 and CLK1 over the other tested enzymes. Molecular modelling proved to be a very useful tool to understand the role of the heteroaryl substituents in the affinity against the panel of tested kinases. Functionalization of position 8 of pyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines with 2,4-disubstituted phenyl groups induced an increased binding affinity with the ATP binding sites of CK1 and CLK1. Contrariwise, substitution at position 7 led to a steric clash on the P-loop of CK1 resulting in a complete loss of inhibitory activity against CK1 while partially sparing the activity on CLK1.

6. Experimental section

6.1. Chemistry

Melting points of powder compounds were measured on an STUART-Advanced apparatus. IR spectra were recorded on a PerkinElmer Spectrum 100 Series FT-IR spectrometer. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker DXP 300 spectrometer at 300, 75 MHz, respectively and a Bruker AVANCE 400 MHz high resolution NMR spectrometer at 400, 100 MHz, respectively. Multiplicities were abbreviated as follows: singlet (s), doublet (d), triplet (t), multiplet (m), and broad (br). Reactions were monitored by TLC analysis using Merck silica gel 60F-254 thin layer plates. Column chromatography was carried out on silica gel Merck 60 (70–230 mesh ASTM). Elemental analyses were found within ±0.4% of the

Table 2
Kinase activity (IC_{50}) of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (**1a**–**q**).[a,b]

Entry	Compound	CDK5/p25	CK1δ/ε	CLK1	DYRK1A	GSK3α/β
1	**1a**	>10	0.63	**0.19**	11	>10
2	**1b**	>10	>10	2.5	>10	>10
3	**1c**	>10	1.1	**0.28**	>10	>10
4	**1d**	>10	0.51	1.1	1.1	>10
5	**1e**	>10	0.91	0.69	1.9	>10
6	**1f**	>10	0.88	2.4	>10	>10
7	**1g**	>10	**0.22**	**0.088**	0.53	>10
8	**1h**	>10	1.2	1.7	6.8	>10
9	**1i**	>10	**0.22**	**0.13**	1.1	>10
10	**1j**	>10	**0.22**	**0.11**	1.4	>10
11	**1k**	>10	**0.38**	**0.19**	**0.33**	>10
12	**1l**	>10	**0.5**	**0.071**	2	>10
13	**1m**	>10	1.1	0.91	2.1	>10
14	**1n**	>10	0.64	**0.5**	1.4	>10
15	**1o**	>10	0.53	0.52	3.8	>10
16	**1p**	>10	**0.19**	**0.18**	1.3	>10
17	**1q**	>10	0.57	0.95	1.3	>10
18	**10**	>10	>10	0.1	6.1	>10
19	**II**[c]	>10	**0.031**	0.68	11	>10
20	**Harmine**[d]	>10	1.5	**0.026**	**0.029**	>10

[a] IC_{50} values are reported in μM. The most significant results ($IC_{50} \leq 0.5$ μM) are presented in bold.
[b] Kinases activities were assayed in triplicate. Typically, the standard deviation of single data points was below 10%.
[c] Data given in Ref. [6b].
[d] Data given in Ref. [21].

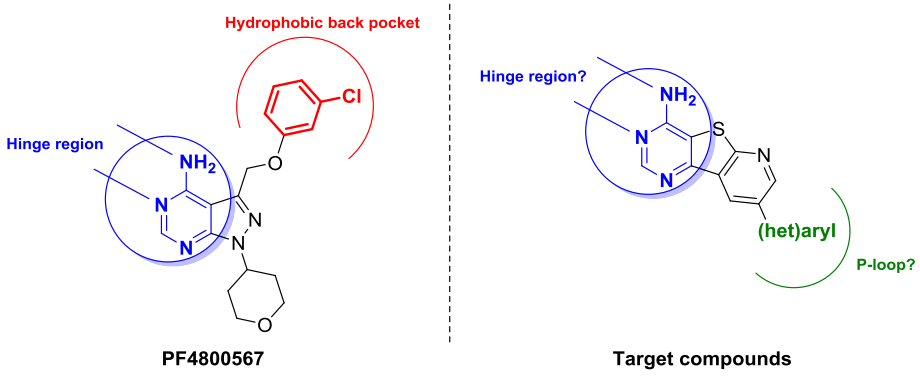


Fig. 1. Structures of CK1 inhibitor PF4800567 (a) and the design concept of 8-(het)arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines (b).

theoretical values. Mass spectra were performed by the Mass Spectrometry Laboratory of the University of Rouen. Mass spectra (EI) were recorded with a Waters ZQ 2000 and a Waters LCP 1er XR spectrometer.

Microwave experiments were conducted in a commercial microwave reactor especially designed for synthetic chemistry. RotoSYNTH™ (Milestone S.r.l. Italy) is a multi-mode cavity with a microwave power delivery system ranging from 0 to 1200 W. The temperatures of the reactions were mainly monitored *via* contactless infrared pyrometer which was calibrated in control experiments with a fibre-optic contact thermometer protected in a Teflon coated ceramic well inserted directly in the reaction mixture. Open vessel experiments were carried out in a 50–250 mL round bottom flask fitted with a reflux condenser. The vessel contents were stirred by means of an adjustable rotating magnetic plate located below the floor of the microwave cavity and a Teflon-coated magnetic stir bar inside the vessel. Temperature and power profiles were monitored in both cases through the EASY-Control software provided by the manufacturer. Time indicated in the various protocols is the time measured when the mixtures reached the programmed temperature after a ramp period of 2 min.

Compounds **1a**–**g** and their precursors (**2**, **3**, **4** and **5a**–**g**) were described in a previous paper [12a].

*6.1.1. General procedure for the synthesis of (E)-N′-(5-aryl-2-cyano) thieno[2,3-b]pyridin-3-yl)-N,N-dimethylformimidamide derivatives (**5h**–**q**)*

A mixture of (*E*)-*N*′-(5-bromo-2-cyanothieno[2,3-*b*]pyridin-3-yl)-*N*,*N*-dimethyl formimi-damide **4** (0.160 g, 0.52 mmol), appropriate boronic acid (1.5 equiv), sodium carbonate (0.110 g, 1.04 mmol) and $Pd(PPh_3)_4$ (0.016 g) in DMF (8 mL) was irradiated at 150 °C (800 W) for 90 min. On completion (followed by thin-layer chromatography), the reaction was cooled to room temperature and H_2O was added. The powder was filtered off, washed with EtOAc and the organic layer was evaporated in *vacuo*. The crude powder was purified by silica gel column chromatography using a gradient of PE/EtOAc (100:0 to 0:100, v/v) as the eluent to give the desired compounds.

*6.1.1.1. (E)-N′-(2-cyano-5-(4-(trifluoromethyl)phenyl)thieno[2,3-b] pyridin-3-yl)-N,N-dimethylformimidamide (**5h**).* Yellow powder (0.124 g, 64%); mp 146–148 °C; IR ν_{max} (cm^{-1}): 2201 (CN), 1628, 1616, 1588, 1537, 1486, 1416, 1386, 1362, 1329, 1251, 1167, 1107, 1072, 1050, 1014, 983, 912, 836, 761; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (d, 1H, $J = 2$ Hz, *H*-4), 8.46 (d, 1H, $J = 2$ Hz, *H*-6), 8.31 (s, 1H, NC*H*N), 8.07 (d, 2H, $J = 9$ Hz, *H*-ar), 7.88 (d, 2H, $J = 9$ Hz, *H*-ar), 3.16 (s, 3H, NCH_3), 3.11 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.9, 156.3, 155.3, 149.7, 140.6, 131.4, 129.8, 128.8, 128.3 (2C), 128.2, 127.9, 125.9 (2C), 115.7, 86.0, 34.3 (2C); HRMS calcd for $C_{18}H_{14}F_3N_4S$ $[M+H]^+$ 375.0891 found 375.0886.

*6.1.1.2. (E)-N′-(5-(2,4-Difluorophenyl)-2-cyanothieno[2,3-b]pyridin-3-yl)-N,N-dimethyl-formimidamide (**5i**).* White powder (0.125 g, 70%); mp 215–217 °C; IR ν_{max} (cm^{-1}): 2197 (CN), 1632, 1615, 1590, 1540, 1512, 1421, 1359, 1266, 1145, 1111, 1097, 1044, 974, 966, 962, 847, 730; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (d, 1H, $J = 2$ Hz, *H*-4), 8.32 (s, 1H, NC*H*N), 8.32 (d, 1H, $J = 2$ Hz, *H*-6), 7.82–7.77 (m, 1H, *H*-ar), 7.54–7.46 (m, 1H, *H*-ar), 7.34–7.28 (m, 1H, *H*-ar), 3.19 (s, 3H, NCH_3), 3.13 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 156.4, 155.2, 150.7, 132.5, 131.5, 127.7, 127.2, 115.7, 112.6, 112.3, 105.0, 104.7, 104.3, 85.7, 34.2 (2C); HRMS calcd for $C_{17}H_{13}F_2N_4S$ $[M+H]^+$ 343.0829 found 343.0820.

*6.1.1.3. (E)-N′-(5-(4-Chloro-2-fluorophenyl)-2-cyanothieno[2,3-b] pyridin-3-yl)-N,N-dimethyl formimidamide (**5j**).* Pale yellow powder (0.125 g, 67%); mp 222–224 °C; IR ν_{max} (cm^{-1}): 2189 (CN), 1626,

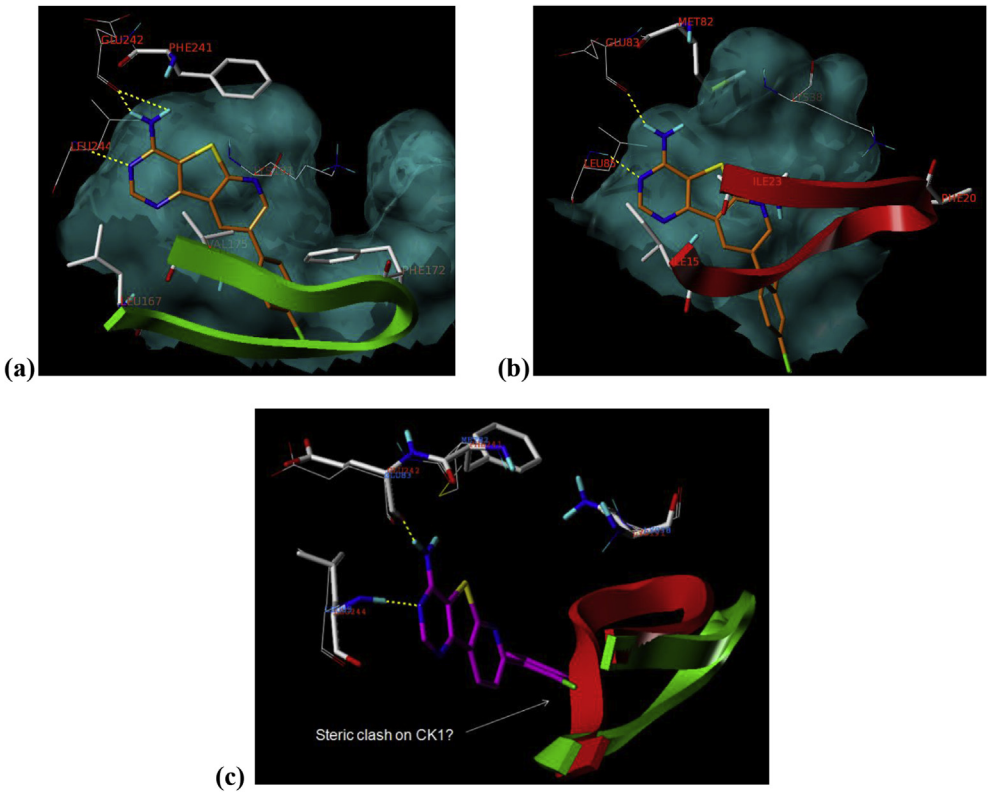


Fig. 2. (a) Docking model of compound **1g** into the ATP site of CLK1; (b) Docking model of compound **1g** into the ATP site of CK1; (c) Docking model of compound **10** into the ATP site of CLK1 superimposed with CK1. Active site pocket is highlighted in cyan (MOLCAD surface; program SYBYL-X 1.3). Hydrogens bonds are indicated as yellow dotted lines. P-loops are presented as green (CLK1) or red (CK1) ribbons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1613, 1584, 1537, 1473, 1458, 1414, 1405, 1386, 1370, 1264, 1248, 1210, 1108, 1037, 979, 894, 925, 759; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.93 (t, 1H, J = 2 Hz, H-4), 8.32 (t, 1H, J = 2 Hz, H-6), 8.29 (s, 1H, NCHN), 7.76 (dd, 1H, J_1 = 8 Hz, J_2 = 9 Hz, H-ar), 7.65 (dd, 1H, J_1 = 2 Hz, J_2 = 9 Hz, H-ar), 7.47 (dd, 1H, J_1 = 2 Hz, J_2 = 8 Hz, H-ar), 3.15 (s, 3H, NCH$_3$), 3.09 (s, 3H, NCH$_3$); HRMS calcd for $C_{17}H_{13}ClFN_4S$ $[M+H]^+$ 359.0533 found 359.0548.

*6.1.1.4. (E)-N′-(5-(2-Chloro-4-fluorophenyl)-2-cyanothieno[2,3-b] pyridin-3-yl)-N,N-dimethyl-formimidamide (**5k**).* Pale yellow powder (0.163 g, 88%); mp 208–210 °C; IR ν_{max} (cm^{-1}): 2201 (CN), 1628, 1600, 1588, 1544, 1539, 1503, 1476, 1462, 1382, 1361, 1254, 1205, 1108, 1043, 969, 893, 866, 825; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (d, 1H, J = 2 Hz, H-4), 8.27 (s, 1H, NCHN), 8.23 (d, 1H, J = 2 Hz, H-6), 7.68–7.62 (m, 2H, H-ar), 7.39 (td, 1H, J_1 = 3 Hz, J_2=9 Hz, H-ar), 3.13 (s, 3H, NCH$_3$), 3.06 (s, 3H, NCH$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 156.4, 155.2, 151.3, 133.5, 132.8, 132.2, 130.7, 127.4, 117.3, 116.9, 115.8, 115.2, 114.8, 85.8, 34.4 (2C); HRMS calcd for $C_{17}H_{13}ClFN_4S$ $[M+H]^+$ 359.0533 found 359.0537.

*6.1.1.5. (E)-N′-(5-(2-Fluoro-4-methoxyphenyl)-2-cyanothieno[2,3-b] pyridin-3-yl)-N,N-dimethylformimidamide (**5l**).* White powder (0.184 g, 99%); mp 180–182 °C; IR ν_{max} (cm^{-1}): 2201 (CN), 1617, 1589, 1536, 1478, 1461, 1425, 1382, 1365, 1292, 1263, 1164, 1130, 1114, 1030, 992, 913, 860, 810; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.90 (t, 1H, J = 2 Hz, H-4), 8.29 (s, 1H, NCHN), 8.26 (t, 1H, J = 2 Hz, H-6), 7.64 (dd, 1H, J_1 = 8 Hz, J_2 = 9 Hz, H-ar), 7.07–6.95 (m, 2H, H-ar), 3.85 (s, 3H, OCH$_3$), 3.15 (s, 3H, NCH$_3$), 3.09 (s, 3H, NCH$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.1, 157.8, 156.4, 155.0, 150.7, 131.8, 131.0, 128.0, 127.7, 116.5, 115.7, 111.3, 102.3, 102.0, 85.7, 55.9, 34.5 (2C); HRMS calcd for $C_{18}H_{16}FN_4OS$ $[M+H]^+$ 355.1029 found 355.1027.

*6.1.1.6. (E)-N′-(5-(4-Bromophenyl)-2-cyanothieno[2,3-b]pyridin-3-yl)-N,N-dimethylformimi-damide (**5m**).* White powder (0.028 g, 14%); mp 84–186 °C; IR ν_{max} (cm^{-1}): 2188 (CN), 1625, 1536, 1476, 1458, 1408, 1362, 1249, 1108, 1008, 978, 914, 870, 818, 759; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (d, 1H, J = 2 Hz, H-4), 8.39 (d, 1H, J = 2 Hz, H-6), 8.30 (s, 1H, NCHN), 7.81 (d, 2H, J = 9 Hz, H-ar), 7.73 (d, 2H, J = 9 Hz, H-ar), 3.16 (s, 3H, NCH$_3$), 3.11 (s, 3H, NCH$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 156.4, 155.5, 149.6, 135.7, 132.0 (2C), 131.7, 129.4, 129.3 (2C), 127.9, 121.9, 115.8, 86.0, 34.3 (2C); HRMS calcd for $C_{17}H_{14}BrN_4S$ $[M+H]^+$ 385.0123 found 385.0124.

*6.1.1.7. (E)-N′-(5-(4-Nitrophenyl)-2-cyanothieno[2,3-b]pyridin-3-yl)-N,N-dimethylformimi-damide (**5n**).* Yellow powder (0.041 g, 22%); mp 208–210 °C; IR ν_{max} (cm^{-1}): 2196 (CN), 1627, 1591, 1545, 1515, 1480, 1464, 1437, 1359, 1343, 1257, 1093, 1015, 975, 919, 868, 851, 792, 759, 753, 737; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (d, 1H, J = 2 Hz, H-4), 8.57 (d, 1H, J = 2 Hz, H-6), 8.43 (d, 2H, J = 9 Hz, H-ar), 8.38 (s, 1H, NCHN), 8.21 (d, 2H, J = 9 Hz, H-ar), 3.22 (s, 3H, NCH$_3$), 3.18 (s, 3H, NCH$_3$); HRMS calcd for $C_{17}H_{14}N_5O_2S$ $[M+H]^+$ 352.0868 found 352.0868.

*6.1.1.8. (E)-N′-(5-(Benzo[d][1,3]dioxol-5-yl)-2-cyanothieno[2,3-b] pyridin-3-yl)-N,N-dimethyl formimidamide (**5o**).* Yellow powder (0.116 g, 64%); mp 194–196 °C; IR ν_{max} (cm^{-1}): 2182 (CN), 1621, 1471, 1446, 1369, 1324, 1252, 1229, 1109, 1097, 1052, 1034, 1017, 977, 931, 870, 859, 799, 756; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.03 (d, 1H, J = 2 Hz, H-4), 8.30 (d, 1H, J = 2 Hz, H-6), 8.29 (s, 1H, NCHN), 7.45 (d, 1H, J = 2 Hz, H-ar), 7.30 (dd, 1H, J_1 = 2 Hz, J_2 = 8 Hz, H-ar), 7.07 (d, 1H, J = 8 Hz, H-ar), 6.11 (s, 2H, CH$_2$), 3.15 (s, 3H, NCH$_3$), 3.11 (s, 3H, NCH$_3$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.6, 156.2, 155.3, 149.6, 148.2, 147.5, 132.8, 130.5, 128.8, 127.8, 121.2, 115.8, 108.9, 107.7, 101.4,

85.7, 34.2 (2C); HRMS calcd for $C_{18}H_{15}N_4O_2S$ $[M+H]^+$ 351.0916 found 351.0909.

*6.1.1.9. (E)-N′-(2-Cyano-5-(thiophen-2-yl)thieno[2,3-b]pyridin-3-yl)-N,N-dimethylformimi- damide (**5p**).* Light brown powder (0.128 g, 79%); mp 175–176 °C; IR ν_{max} (cm^{-1}): 2191 (CN), 1627, 1539, 1527, 1482, 1467, 1409, 1387, 1370, 1353, 1304, 1259, 1237, 1114, 1035, 980, 901, 849, 755; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.12 (d, 1H, J = 2 Hz, H-4), 8.29 (d, 1H, J = 2 Hz, H-6), 8.28 (s, 1H, NCHN), 7.79 (dd, 1H, J_1 = 1 Hz, J_2 = 3 Hz, H-ar), 7.70 (dd, 1H, J_1 = 1 Hz, J_2 = 5 Hz, H-ar), 7.23 (dd, 1H, J_1 = 3 Hz, J_2 = 5 Hz, H-ar), 3.16 (s, 3H, NCH_3), 3.12 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.6, 156.3, 155.1, 148.3, 138.9, 128.8, 128.0, 127.2 (2C), 127.1, 125.7, 115.7, 85.9, 34.3 (2C); HRMS calcd for $C_{15}H_{13}N_4S_2$ $[M+H]^+$ 313.0568 found 313.0581.

*6.1.1.10. (E)-N′-(2-Cyano-5-(pyrimidin-5-yl)thieno[2,3-b]pyridin-3-yl)-N,N-dimethylformimi damide (**5q**).* Grey powder (0.087 g, 54%); mp 229–230 °C; IR ν_{max} (cm^{-1}): 2200 (CN), 1625, 1536, 1462, 1415, 1384, 1258, 1117, 1044, 989, 900, 873, 759, 724; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.32 (d, 2H, J = 1 Hz, H-ar), 9.28 (d, 1H, J = 1 Hz, H-ar), 9.20 (d, 1H, J = 2 Hz, H-4), 8.62 (d, 1H, J = 2 Hz, H-6), 8.32 (s, 1H, NCHN), 3.16 (s, 3H, NCH_3), 3.12 (s, 3H, NCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.3, 157.8, 156.3, 155.4 (2C), 155.3, 149.5, 130.3, 130.2, 127.8, 127.0, 115.7, 86.1, 34.2 (2C); HRMS calcd for $C_{15}H_{13}N_6S$ $[M+H]^+$ 309.0922 found 309.0931.

*6.1.2. General procedure for the synthesis of 8-arylpyrido [3′,2′:4,5] thieno[3,2-d]pyrimidin-4-amines (**1h**–**q**)*

Formamide (2.0 mL) was added to the corresponding formimidamide (0.20 mmol). The mixture was irradiated at various temperature (200 W) for 30 min. On completion (followed by GC–MS chromatography), the reaction was cooled to room temperature and H_2O was added. The powder was filtered off, washed with H_2O and dried. The crude powder was purified by silica gel column chromatography using DCM/EtOAc (100:0 to 0:100, v/v) as the eluent to give the desired compounds.

*6.1.2.1. 8-(4-(Trifluoromethyl)phenyl)pyrido[3′,2′:4,5]thieno [3,2-d] pyrimidin-4-amine (**1h**).* Kaki powder (0.044 g, 64%), mp > 300 °C; IR ν_{max} (cm^{-1}): 3137, 1627, 1558, 1543, 1517, 1478, 1317, 1288, 1268, 1251, 1165, 1111, 1066, 1042, 1014, 962, 862, 843, 787, 778; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (d, 1H, J = 2 Hz, H-9), 8.90 (d, 1H, J = 2 Hz, H-7), 8.60 (s, 1H, H-2), 8.14 (d, 2H, J = 8 Hz, H-ar), 7.90 (d, 2H, J = 8 Hz, H-ar), 7.74 (br s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.0, 158.5, 155.6, 153.3, 150.2, 140.7, 131.5, 129.2, 128.8, 128.3 (2C), 128.1, 127.9, 126.0 (2C), 113.1; HRMS calcd for $C_{16}H_{10}F_3N_4S$ $[M+H]^+$ 347.0578 found 347.0581.

*6.1.2.2. 8-(2,4-Difluorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1i**).* Yellow powder (0.042 g, 66%), mp > 300 °C; IR ν_{max} (cm^{-1}): 3111, 1663, 1596, 1572, 1544, 1529, 1514, 1422, 1372, 1313, 1287, 1271, 1257, 1228, 1153, 955, 921, 885, 810, 786, 731; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.98 (d, 1H, J = 2 Hz, H-9), 8.71 (d, 1H, J = 2 Hz, H-7), 8.57 (s, 1H, H-2), 7.85 (ddd, 1H, J 1, 2, 8 Hz, H-ar), 7.74 (br s, 2H, NH_2), 7.48 (ddd, 1H, J 1, 3, 8 Hz, H-ar), 7.27 (ddd, 1H, J 1, 2, 3 Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.5, 159.0, 155.5, 153.3, 151.2, 132.5, 130.7, 127.7, 127.2, 121.3, 113.0, 112.6, 112.3, 105.1, 104.8; HRMS calcd for $C_{15}H_9F_2N_4S$ $[M+H]^+$ 315.0516 found 315.0524.

*6.1.2.3. 8-(4-Chloro-2-fluorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d] pyrimidin-4-amine (**1j**).* Light brown powder (0.043 g, 66%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3107, 1672, 1568, 1542, 1525, 1497, 1466, 1396, 1371, 1310, 1282, 1260, 1243, 1208, 1086, 899, 855, 812, 785; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.00 (d, 1H, J = 2 Hz, H-9), 8.74 (d, 1H, J = 2 Hz, H-7), 8.59 (s, 1H, H-2), 7.82 (ddd, 1H, J 1, 2, 8 Hz, H-ar), 7.75 (br s, 2H, NH_2), 7.66 (dd, 1H, J 3, 8 Hz, H-ar), 7.49 (dd, 1H, J 2, 3 Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.8, 158.5, 155.7, 153.2, 151.2, 134.1, 132.3, 130.9, 127.8, 127.0, 125.6, 123.8, 117.1, 116.6, 113.1; HRMS calcd for $C_{15}H_9ClFN_4S$ $[M+H]^+$ 331.0220 found 331.0238.

*6.1.2.4. 8-(2-Chloro-4-fluorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d] pyrimidin-4-amine (**1k**).* Brown powder (0.052 g, 79%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3118, 1658, 1574, 1503, 1392, 1250, 1205, 902, 852, 785; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.87 (d, 1H, J = 2 Hz, H-9), 8.63 (d, 1H, J = 2 Hz, H-7), 8.58 (s, 1H, H-2), 7.76–7.67 (m, 4H, H-ar and NH_2), 7.43 (dd, 1H, J 2, 3 Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.5, 158.5, 155.6, 153.1, 151.7, 133.4, 132.5, 131.6, 130.7, 127.2, 117.5, 117.1, 115.2, 114.9, 113.0; HRMS calcd for $C_{15}H_9ClFN_4S$ $[M+H]^+$ 331.0220 found 331.0227.

*6.1.2.5. 8-(2-Fluoro-4-methoxyphenyl)pyrido[3′,2′:4,5]thieno [3,2-d] pyrimidin-4-amine (**1l**).* Light brown powder (0.044 g, 61%); mp 296–298 °C; IR ν_{max} (cm^{-1}): 3121, 1650, 1627, 1594, 1570, 1542, 1519, 1468, 1372, 1317, 1297, 1252, 1226, 1162, 1133, 1073, 1047, 860, 786; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.97 (d, 1H, J = 2 Hz, H-9), 8.66 (d, 1H, J = 2 Hz, H-7), 8.58 (s, 1H, H-2), 7.74 (br s, 2H, NH_2), 7.70 (ddd, 1H, J 1, 2, 9 Hz, H-ar), 7.05 (dd, 1H, J 4, 9 Hz, H-ar), 6.98 (dd, 1H, J 2, 4 Hz, H-ar), 3.86 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.0, 159.8, 158.6, 155.7, 153.2, 151.1, 131.5, 130.5, 128.1, 127.5, 116.4, 113.2, 111.3, 102.5, 102.1, 55.9; HRMS calcd for $C_{16}H_{12}FN_4OS$ $[M+H]^+$ 327.0716 found 327.0703.

*6.1.2.6. 8-(4-Bromophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1m**).* Light brown powder (0.046 g, 65%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3142, 1674, 1576, 1540, 1520, 1371, 1249, 1077, 1008, 862, 813, 785, 762; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.16 (d, 1H, J = 2 Hz, H-9), 8.83 (d, 1H, J = 2 Hz, H-7), 8.60 (s, 1H, H-2), 7.88 (d, 2H, J = 8 Hz, H-ar), 7.76 (br s, 2H, NH_2), 7.75 (d, 2H, J = 8 Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.6, 158.5, 155.5, 153.5, 150.0, 135.9, 132.2 (2C), 131.9, 129.3 (2C), 128.6, 128.0, 121.9, 102.3; HRMS calcd for $C_{15}H_{10}BrN_4S$ $[M+H]^+$ 356.9810 found 356.9805.

*6.1.2.7. 8-(4-Nitrophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1n**).* Light brown powder (0.036 g, 55%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3112, 1660, 1568, 1518, 1373, 1342, 1270, 1248, 1103, 1071, 1027, 913, 847, 784, 753; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (d, 1H, J = 2 Hz, H-9), 8.95 (d, 1H, J = 2 Hz, H-7), 8.60 (s, 1H, H-2), 8.36 (d, 2H, J = 8 Hz, H-ar), 8.21 (d, 2H, J = 8 Hz, H-ar), 7.78 (br s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 155.7, 153.3, 147.2, 143.1, 132.6, 130.9, 129.4, 128.6, 127.9, 124.2, 120.8, 119.7, 118.1, 113.2; HRMS calcd for $C_{15}H_{10}N_5O_2S$ $[M+H]^+$ 324.0555 found 324.0540.

*6.1.2.8. 8-(Benzo[d][1,3]dioxol-5-yl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1o**).* Light brown powder (0.060 g, 93%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3137, 1627, 1572, 1539, 1509, 1470, 1444, 1377, 1243, 1111, 1073, 1037, 978, 931, 806, 785; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (d, 1H, J = 2 Hz, H-9), 8.74 (d, 1H, J = 2 Hz, H-7), 8.59 (s, 1H, H-2), 7.72 (br s, 2H, NH_2), 7.51 (d, 1H, J = 1 Hz, H-ar), 7.37 (dd, 1H, J_1 = 1 Hz, J_2 = 8 Hz, H-ar), 7.08 (d, 1H, J = 8 Hz, H-ar), 6.12 (br s, 2H, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 155.4, 153.3, 150.0, 148.2, 147.6, 132.8, 130.6, 128.2, 127.8, 126.1, 121.1, 113.1, 108.9, 107.6, 101.4; HRMS calcd for $C_{16}H_{11}N_4O_2S$ $[M+H]^+$ 323.0603 found 323.0599.

*6.1.2.9. 8-(4-Nitrophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1p**).* Brown powder (0.049 g, 87%); mp 254–255 °C; IR ν_{max} (cm^{-1}): 1636, 1572, 1545, 1520, 1475, 1436, 1427, 1379, 1291, 1258, 1207, 898, 786, 745, 709; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.18 (d,

1H, $J = 2$ Hz, H-9), 8.74 (d, 1H, $J = 2$ Hz, H-7), 8.59 (s, 1H, H-2), 7.68 (dd, 1H, J_1=2 Hz, $J_2 = 5$ Hz, H-ar), 7.76–7.73 (m, 3H, H-ar and NH_2), 7.26 (d, 1H, $J = 2$ Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.6, 158.5, 155.5, 153.0, 148.5, 139.0, 128.9, 127.8, 127.1 (2C), 126.6, 125.6, 113.2; HRMS calcd for $C_{13}H_9N_4S_2$ $[M+H]^+$ 285.0269 found 285.0277.

*6.1.2.10. 8-(Pyrimidin-5-yl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**1q**).* Light brown powder (0.038 g, 67%); mp > 300 °C; IR ν_{max} (cm^{-1}): 1652, 1578, 1417, 1376, 1254, 1129, 1077, 908, 864, 787, 723; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.39 (d, 2H, $J = 1$ Hz, H-ar), 9.29 (s, 1H, $J = 1$ Hz, H-ar), 9.27 (d, 1H, $J = 2$ Hz, H-9), 9.04 (d, 1H, $J = 2$ Hz, H-7), 8.61 (s, 1H, H-2), 7.79 (br s, 2H, NH_2); HRMS calcd for $C_{13}H_9N_6S$ $[M+H]^+$ 281.0609 found 281.0598.

*6.1.3. Synthesis of 7-(2,4-dichlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**10**)*

*6.1.3.1. 2-Chloro-6-(2,4-dichlorophenyl)nicotinonitrile (**7**).* A mixture of 2,6-dichloropyridine-3-carbonitrile (**6**) (0.1 g, 0.58 mmol), 2,4-dichlorophenylboronic acid (0.12 g, 0.63 mmol), sodium carbonate (0.184 g, 1.73 mmol) and $Pd(PPh_3)_4$ (0.034 g) in dioxane (3 mL)/H_2O (1 mL) was irradiated at 100 °C (400 W) for 2 h. On completion (followed by thin-layer chromatography), the reaction was cooled to room temperature and H_2O was added. The powder was filtered off, washed with EtOAc and the organic layer was evaporated in *vacuo*. The crude powder was purified by silica gel column chromatography using a gradient of PE/EtOAc (100:0 to 0:100, v/v) as the eluent to give the desired compound **7** as a yellow powder (0.143 g, 87%); mp 188–189 °C; IR ν_{max} (cm^{-1}): 2238 (CN), 1587, 1578, 1556, 1432, 1349, 1300, 1172, 1142, 1090, 1043, 1030, 881, 869, 850, 817, 792; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.62 (d, 1H, $J = 8$ Hz, H-4), 7.98 (d, 1H, $J = 8$ Hz, H-5), 7.86 (d, 1H, $J = 2$ Hz, H-ar), 7.69 (d, 1H, $J = 9$ Hz, H-ar), 7.63 (dd, 1H, J_1=2 Hz, $J_2 = 9$ Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.5, 150.7, 144.4, 135.5, 134.8, 133.0, 132.2, 129.7, 128.0, 124.0, 115.0, 108.8; HRMS calcd for $C_{12}H_6Cl_3N_2$ $[M+H]^+$ 282.9597 found 282.9598.

*6.1.3.2. 3-Amino-6-(2,4-dichlorophenyl)thieno[2,3-b]pyridine-2-carbonitrile (**8**).* A solution of potassium hydroxide (82 mg, 1.46 mmol) in H_2O (0.4 mL) was added dropwise to a stirred and cold solution (ice bath) containing 2-chloro-6-(2,4-dichlorophenyl) nicotinonitrile (**7**) (0.138 g, 0.49 mmol) and 3-mercaptopropionitrile (0.051 g, 0.58 mmol) in DMF (4 mL). The cold mixture was stirred for 15 min and bromoacetonitrile (52 μL, 0.73 mmol) was added dropwise. After 1 h at 0 °C, the mixture was poured onto iced water. The crude product was collected by filtration and purified with silica gel column chromatography using a gradient of PE/EtOAc (100:0 to 0:100, v/v) as the eluent to give the desired compound **8** as a yellow powder (0.043 g, 28%); mp (neat) 285–286 °C; IR (neat) ν_{max} (cm^{-1}): 2192 (CN), 1641, 1577, 1532, 1477, 1449, 1407, 1383, 1347, 1209, 1142, 1107, 1091, 1031, 861, 839, 813, 772; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.61 (d, 1H, $J = 8$ Hz, H-4), 7.80 (m, 2H, H-5 and H-ar), 7.69 (d, 1H, $J = 9$ Hz, H-ar), 7.59 (dd, 1H, J_1=2 Hz, $J_2 = 9$ Hz, H-ar), 7.43 (br s, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.2, 156.3, 150.2, 136.8, 134.5, 133.0, 132.3, 131.5, 129.5, 127.7, 123.3, 121.0, 115.6, 71.8; HRMS calcd for $C_{14}H_8Cl_2N_3S$ $[M+H]^+$ 319.9816 found 319.9812.

*6.1.3.3. (E)-N′-(2-Cyano-6-(2,4-dichlorophenyl)thieno[2,3-b]pyridin-3-yl)-N,N-dimethyl formimidamide (**9**).* A mixture of 3-amino-6-(2,4-dichlorophenyl)thieno[2,3-*b*]pyridine-2-carbonitrile **8** (0.066 g, 0.20 mmol) and DMF-DMA (2 mL) was irradiated at 90 °C (800 W) for 30 min. On completion, the solution was cooled to room temperature and crude products were extracted with EtOAc. The organic layers were washed with cold H_2O, dried over Na_2SO_4, filtered and evaporated in *vacuo*. Purification by silica gel column chromatography using PE/EtOAc (5:5, v/v) as the eluent gave the desired compound **9** as a yellow powder (0.056 g, 73%); mp 216–218 °C; IR ν_{max} (cm^{-1}): 2195 (CN), 1631, 1574, 1558, 1477, 1434, 1415, 1378, 1340, 1100, 1083, 1039, 1028, 975, 852, 843, 820, 769; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.35 (d, 1H, $J = 8$ Hz, H-4), 8.29 (s, 1H, NCHN), 7.81 (m, 2H, H-5 and H-ar), 7.71 (d, 1H, $J = 8$ Hz, H-ar), 7.61 (dd, 1H, J_1=2 Hz, $J_2 = 8$ Hz, H-ar), 3.16 (s, 3H, CH_3), 3.10 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.9, 159.0, 156.4, 155.1, 136.8, 134.6, 133.1, 132.4, 132.3, 129.6, 127.8, 126.9, 121.7, 115.9, 85.9, 34.2 (2C); HRMS calcd for $C_{17}H_{13}Cl_2N_4S$ $[M+H]^+$ 375.0238 found 375.0235.

*6.1.3.4. 7-(2,4-Dichlorophenyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4-amine (**10**).* Formamide (2 mL) was added to (*E*)-*N*′-(2-cyano-6-(2,4-dichlorophenyl)thieno[2,3-*b*]pyridin-3-yl)-*N*,*N*-dimethylformimidamide (**9**) (0.050 g, 0.07 mmol). The mixture was irradiated at 185 °C (200 W) for 1 h. On completion (followed by GC–MS chromatography), the reaction was cooled to room temperature and H_2O was added. The powder was filtered off, washed with H_2O and dried. The crude powder was purified by silica gel column chromatography using DCM/EtOAc (100:0 to 0:100, v/v) as the eluent to give the desired compound **10** as a pale yellow powder (0.038 g, 83%); mp > 300 °C; IR ν_{max} (cm^{-1}): 3136, 1664, 1575, 1523, 1477, 1442, 1402, 1382, 1340, 1291, 1105, 1089, 1029, 868, 844, 787, 757; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.73 (d, 1H, $J = 8$ Hz, H-9), 8.59 (s, 1H, H-2), 7.92 (d, 1H, $J = 8$ Hz, H-8), 7.85 (d, 1H, $J = 2$ Hz, H-ar), 7.78 (m, 3H, H-ar and NH_2), 7.63 (dd, 1H, $J_1 = 2$ Hz, $J_2 = 8$ Hz, H-ar); ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.9, 158.4, 157.0, 155.6, 136.9, 134.5, 133.2, 132.3, 131.6, 129.5, 127.8, 127.7, 126.9, 121.9, 112.9; HRMS calcd for $C_{15}H_9Cl_2N_4S$ $[M+H]^+$ 346.9925 found 346.9931.

6.2. In vitro kinase preparation and assays [17]

6.2.1. Buffers

Buffer A: $MgCl_2$ (10 mM), 1 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 25 mM Tris–HCl pH 7.5, 50 μg heparin/mL.

Buffer B: β-Glycerophosphate (60 mM), 30 mM p-nitrophenylphosphate, 25 mM 3-(*N*-morpholino)propanesulfonic acid (Mops) (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM sodium vanadate.

6.2.2. Kinase preparations and assays

Kinase activities were assayed in triplicates in buffer A or B, for 30 min at 30 °C, at a final adenosine triphosphate (ATP) concentration of 15 μM. Blank values were substracted and activities expressed in % of the maximal activity, *i.e.*, in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethylsulfoxide (DMSO). IC_{50} values were calculated from dose–response curves established by Sigma-Plots. The GSK-3, CK1, DYRK1A and CLK1 peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

6.2.2.1. CDK5/p25. (Human, recombinant) was prepared as previously described [18]. Its kinase activity was assayed in buffer A, with 1 mg of histone H1/mL, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto sheets of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed eight times (for at least 5 min each time) in a solution of 10 mL phosphoric acid/L of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid.

6.2.2.2. GSK-3α/β. (Porcine brain, native) was assayed, as described

for CDK5/p25 but in buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQpSEDEEE) (pS stands for phosphorylated serine) [19].

6.2.2.3. CK1δ/ε. (Porcine brain, native) was assayed as described for CDK5/p25 but using the CK1-specific peptide substrate RRKHAAIGpSAYSITA [20].

6.2.2.4. DYRK1A. (Rat, recombinant, expressed in *E. coli* as a glutathione transferase (GST) fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed, as described for CDK5/p25 using Woodtide (KKISGRLSPIMTEQ) (1.5 μg/assay) as a substrate.

6.2.2.5. CLK1. (Human, recombinant, expressed in *E. coli* as GST fusion protein) was assayed in buffer A (+0.15 mg BSA/ml) with RS peptide (GRSRSRSRSRSR) (1 μg/assay).

6.3. Molecular modelling

Molecular modelling studies were performed using SYBYL-X 1.3 software [31] running on a Dell precision T3400 workstation. The three-dimensional structure of compounds **1g** and **10** was built from a standard fragments library and optimized using the Tripos force field [32] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. Powell's method available in Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol.Å. The crystal structures of CLK1 in complex with debromohymenialdisine at 1.70 Å resolution (pdb code: 1Z57) [27] and CK1δ in complex with PF4800567 at 2.07 Å resolution (pdb code: 4HNF) [28] were used as templates for docking. The original ligands as wsell as the water molecules were removed from the coordinates set. Flexible docking of compounds **1g** and **10** into ATP-binding site was performed using GOLD software [33]. A distance range of 1.5–3.0 Å between the N^3 atom of inhibitors and the main chain NH of Leu244/Leu85 (CLK1/CK1) from the hinge region was set as a constraint. The most stable docking model was selected according to the best scored conformation predicted by the GoldScore scoring function.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.12.038.

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