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Original article

Synthesis and optimization of an original V-shaped collection of 4-7-disubstituted Pyrido[3,2-*d*]pyrimidines as CDK5 and DYRK1A inhibitors

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ABSTRACT

We here report the synthesis and biological evaluation of an original collection of 4,7-disubstituted pyrido[3,2-*d*]pyrimidines designed as potential kinase inhibitors. The collection was generated from a single starting material, 4,7-dichloropyrido[3,2-*d*]pyrimidine, which afforded the final compounds after two steps: a sequential or one-pot sequence including selective cross coupling reactions in *C*-4, followed by the second cross-coupling in *C*-7. In position *C*-4, a Suzuki-Miyaura type reaction led to mono-substituted derivatives whereas in position *C*-7, synthesis was achieved via a Suzuki or a Buchwald type reaction using commercially available or undescribed boron derivatives. The biological activity of the V-shaped family was measured in protein kinase assays. The structure activity relationship (SAR) revealed that some compounds selectively inhibited DYRK1A and CDK5 without affecting GSK3. Docking studies furnished possible explanations that correlate with the SAR data. The most active compound on the two biological targets was **27** which exhibited the following IC_{50}: 110 nM for CDK5, 24 nM for DYRK1A and only 1.2 μM for GSK3. In the *C*-7 amino subfamily, the best derivative was indubitably compound **48** which led to a near selective action on DYRK1A and a remarkable IC_{50} of 60 nM.

1. Introduction

Protein kinases constitute a large family of structurally related enzymes that are responsible for the control of a wide variety of signal transduction processes within the cell. Deregulation of kinase activities is frequently observed during the development of human diseases such as cancer, diabetes, Alzheimer's disease, and inflammation [1]. Consequently, they have been used as targets to identify pharmacological inhibitors of potential therapeutic interest [2]. Our laboratory is currently dedicated to the synthesis of novel disease-relevant kinase inhibitors, and focusses its efforts on drugs acting on cyclin-dependent kinase 5 (CDK5), glycogen synthase kinases-3 (GSK3) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) [3]. These enzymes were chosen for their involvement in regulation processes including cancer and central nervous system (CNS) related disorders. Their strong sequence homology and high ATP site conservation make the design of selective inhibitors containing a single organic platform challenging.

CDK5 plays a central role in neuronal migration during the development of the central nervous system [4]. GSK3β is involved in embryonic development, protein synthesis, cell proliferation and differentiation, microtubule dynamics, cell motility and apoptosis [5]. DYRK1A encodes a member of the dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs) family. It may play a significant role in a signalling pathway regulating cell proliferation and may be involved in brain development [3]. A recent review established strategies to inhibit this kinase and related the benefits of the drugs in CNS pathologies [6]. In our opinion it could be interesting to possess dual CDK5/DYRK1A or triple CDK5/GSK3β/

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0223-5234/

DYRK1A inhibitors as they may exert synergistic effects in the treatment of CNS diseases.

We previously developed some molecules able to target some of the above-mentioned kinases in the nanomolar range and showed that tetracyclic V-shaped pyradine or pyrazine or indole containing drugs could be used to design dual ATP competitive CDK5 and DYRK1A inhibitors [7].

Nevertheless series **I** optimization showed that the only substituent that was well tolerated on the indole phenyl ring was the 5-hydroxyl group, which generated an unstable quinonimine system. We therefore sought to discover a novel scaffold able to afford us greater insight into SAR towards kinases. We hypothesised that pharmacophore **I** could be transformed by a caesura/closure strategy into a new model of type **II**. In order to validate this hypothesis, we identified the pyrido[3,2-*d*]pyrimidine core as a suitable solution. Fortunately from a homemade library extraction [8], we obtained encouraging biological results using 4,7-disubstituted derivatives of type **IIa** (Fig. 1).

In this paper we report the first additional synthesis to complete the **IIa** library and we present the novel methodologies leading to derivatives **IIb**–**d**. A literature survey revealed that the synthesis and/or biological evaluation of these derivatives had not been previously described. The full results of kinase assays on CDK5, GSK3β and DYRK1A are provided and SAR is discussed. Complete docking studies were achieved and confirm the interactions of the best candidates with the CDK5 and/or DYRK1A kinase binding sites.

2. Chemistry

2.1. Synthesis of V-shaped molecules of type *IIa*

We previously reported the synthesis of compounds **2**–**21** (Table 1) which were obtained by Suzuki cross coupling reactions [8] from 2,7-dichloropyridine **1**. To explore the potency of the series and to develop efficient SAR, we prepared some additional derivatives. In a novel approach, we carried out the synthesis of **23** using a one pot regioselective *C*-4 followed by a *C*-7 cross coupling procedure. Using first 2-formylthiophen-4-yl and then 4-hydroxyphenyl boronic acids, derivative **23** was isolated in a 24% yield. Starting from **24** [8], which was synthesized on a large scale, and from the two commercially available formylated thiophenyl boronic acids, derivatives **25** and **26** were isolated in satisfying yields (68 and 74% respectively) under microwave irradiation after only 10 min. Reduction of the formyl group of **25** using $NaBH_4$ occurred smoothly in a MeOH/DMF mixture at room temperature and the primary alcohol **27** was isolated in a 71% yield (Scheme 1).

As direct reductive aminations of aldehyde **25** in the presence of secondary aliphatic amines failed, we extra-temporally prepared three undescribed boronic acids by reacting chosen amines in the presence of $NaBH(OAc)_3$ with the 2-formyl-4-thiophenyl boronic acid. The crude adducts **28**–**30** were next used in the Suzuki-Miyaura cross coupling reactions without any purification to afford after hydrolysis the final derivatives **31**–**33** in satisfying global yields. The main advantage of this straightforward procedure is that it is achieved without any protective group (Scheme 2).

2.2. Synthesis of V-shaped molecules of type *IIb* and *IIc*

Having the method in hand to carry out cross coupling reactions in *C*-7 by displacement of the chlorine atom, an unknown series of type **IIb** could then be envisioned to pursue SAR optimization. On performing a Buchwald type reaction (Scheme 3) with (hetero)aromatic primary amines we found that the best conditions required microwave irradiation to activate the reaction and the use of the $Pd(PPh_3)_4$/xantphos couple as catalytic system in the presence of K_2CO_3 as base. Under these optimized conditions, derivatives **34**–**45** were also obtained in fairly good yields (Table 2, entries 1–12). Fortunately, under similar conditions, amides were successfully introduced in *C*-7, leading to type **IIc** compounds. The only modifications were the amount of catalytic system (only 5 mol % in $Pd(OAc)_2$) and an increase in the reaction time from 60 min to 70 min.

In order to give more flexibility to the tri-aromatic V-shaped structure, we sought to introduce onto the pyrimidine ring a "kneecap" through a 4-hydroxyaniline moiety. Beginning the synthesis with **1**, a simple S_NAr was carried out with a near stoichiometric amount of 4-hydroxyaniline and led regioselectively to the *C*-4 aminated derivative **51** in a 70% yield. As we were interested in preparing direct analogues of derivatives **2**–**4** which possess essential hydroxyl aryl groups in *C*-7, the residual chlorine atom of **51** was next involved in the previously presented microwave-activated Suzuki-Miyaura cross coupling reaction. Starting from each commercially available hydroxylated phenyl boronic acid, type **IId** compounds **52**–**54** were isolated without any degradation in 66, 60 and 80% yields respectively, despite the presence of oxidation sensitive functions.

3. Protein kinase inhibition assays

All synthesized products were assayed for potential inhibition of three protein kinases: DYRK1A, CDK5/p25 and GSK-3. Analysis of the collection **2**–**22** (Table 1, entries 1–20) shows that the two CDK5 and DYRK1A enzymes were independently targeted by pyridopyrimidine drugs of type **IIa** with little effect on GSK3. The bisphenol derivatives **2**–**5** interacted more favourably whereas the position of the OH groups on Cy_1 and Cy_2 modulated the kinase inhibitions. The Cy_1-OH group was better positioned in *para* (1–3 *vs* 4–5) whereas the Cy_2-OH function could be displaced from the

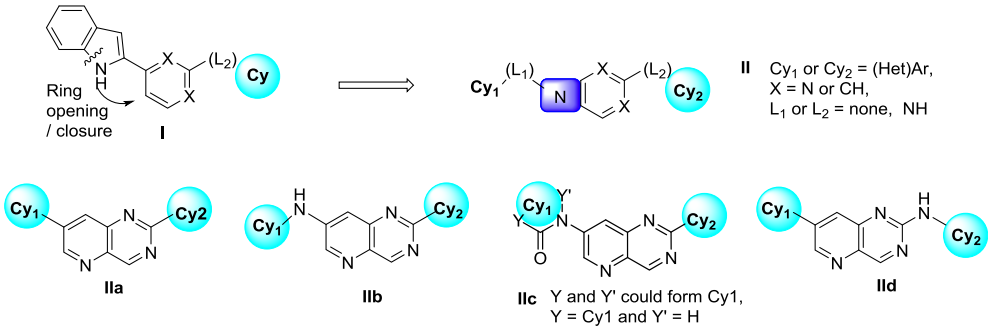
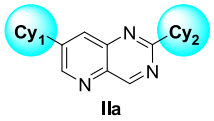
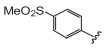


Fig. 1. Scaffold **I** modifications and envisioned library **II**.

Table 1
Full report of the kinase inhibition of the **IIa** library.



IIa

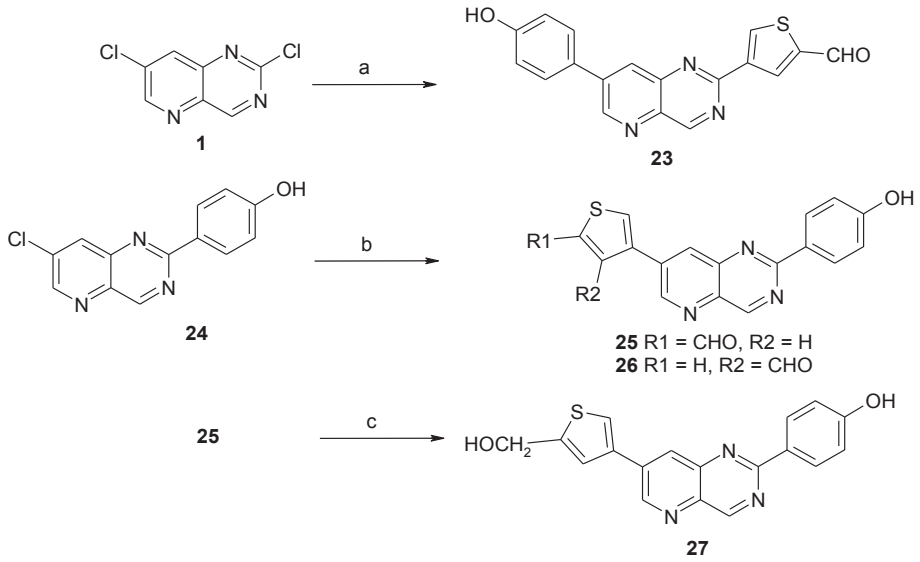
Entry	Compound			Kinase inhibition IC50, μM			Entry	Compound			Kinase inhibition IC50, μM[a]		
	Cy_1	Cy_2		DYRK 1A	CDK5	GSK3		Cy_1	Cy_2		DYRK 1A	CDK5	GSK3
1			**2**	2.7	**0.09**	≥100	15			**16**	>10	0.55	>10
2			**3**	2.4	**0.11**	>100	16			**17**	>10	1.8	>10
3			**4**	0.93	**0.12**	26	17			**18**	>10	**0.11**	>10
4			**5**	0.74	0.72	4	18			**19**	>10	**00.12**	>10
5			**6**	5.2	>10	>10	19			**20**	>10	0.13	>10
6			**7**	24	0.97	≥100	20			**21**	>10	0.65	>10
7			**8**	>10	0.48	>100	21			**22**	>10	1.2	>10
8			**9**	>10	>10	>10	22			**23**	0.38	0.25	>10
9			**10**	>10	>10	>10	23			**25**	0.31	**0.027**	0.43
10			**11**	>10	>10	>10	24			**26**	**0.16**	0.81	3.2
11			**12**	>10	>10	>10	25			**27**	**0.11**	**0.024**	1.2
12			**13**	5.3	0.43	>10	26			**31**	1.4	0.41	0.62
13			**14**	0.88	**0.17**	>10	27			**32**	1	0.3	0.56
14			**15**	**0.15**	0.81	>10	28			**33**	0.97	**0.16**	4.7

[a] Values are means of three measured values of IC_{50}.

ortho to the *para* positions, leading to a clear selectivity for CDK5 active site interaction. Progressive displacement enhanced the potency towards DYRK1A while the effect on CDK5 was stable (compounds **2**–**4**). Selectivity toward GSK3 was affected during these small changes. A decrease in activity was observed using classic phenol isostery. The formyl groups were the only groups to be tolerated by the two kinases and derivatives **13** and **14** were clearly effective as dual DYRK1A and CDK5 inhibitors without any effect on GSK3. As previously mentioned for the OH group, the formyl position modulates the activity profile.

The organoleptic properties of the Cy_2 group were next modified using heterocycles instead of phenol. Pyridine, thiophene and furane skeletons were well tolerated by the CDK5 active site and derivatives **14** and **15** appeared as strong CDK5/DYRK1A inhibitors. Substitutions in *C*-5 thiophene rings which preserved the steric enhancement embraced the kinase active sites. Increasing the size of the substitutions by adding secondary amines led to disappointing results despite the high solubility of derivatives **31**–**33**. In addition, the use of thiophene globally diminished the selectivity towards GSK3. Compounds **25** and **27** remained the most selective derivatives and the best V-shaped CDK5 inhibitors with remarkable IC_{50} values of 27 and 24 nM, respectively.

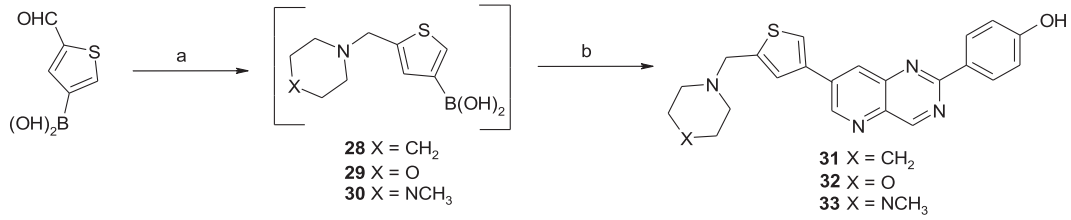
Introduction of an NH or amide *C*-4 or *C*-7 between the pyridopyrimidine and Cy_1 or Cy_2 led respectively to derivatives of type **IIb** and **IId** (Table 2) which were used in kinase assays. In series **IIb**, despite our efforts and the modulation of Cy_1, the only favourable group was the 4-hydroxyphenyl group. The corresponding methyl ether was fully inactive whereas liberation of the hydrogen acid led to **35** (entry 2), which exhibited a sub-micromolar inhibition of DYRK1A and good selectivity towards CDK5 and GSK3. The



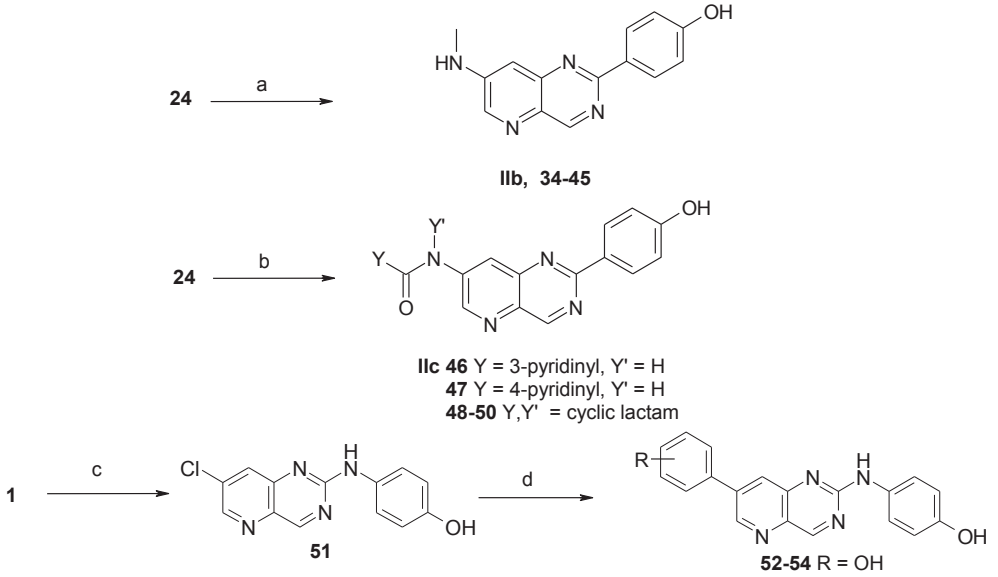
Scheme 1. Preparation of some *C*-2 and *C*-7 thiophene containing derivatives. *Reagents and conditions* : a) 2-formyl-4-thiophenyl boronic acid (1.0 eq.), Na2CO3 (2.0 eq.), Pd(PPh3)4 (5mol %), toluene/EtOH 2/1, 100°C, 6 h and then 4-$OHC_6H4B(OH)2$ (1.2 eq.), 100°C, 1 h, 24% ; b) thiophenyl boron derivative (1.2 eq.), K2CO3 (2.0 eq.), Pd(PPh3)4 (5 mol %), toluene/EtOH 2/1, 150°, mW, 10 min, **25** 68% or **26** 74% ; c) NaBH4 (0.5 eq.), MeOH/DMF 1/1, r.t., 16 h, 71%.

heterocyclic derivatives **36**–**45** were quite inactive on DYRK1A, except for the oxazole derivative **42** which inhibited DYRK1A with an IC_{50} of 2 μM, again indicating the necessary presence of electron rich fractions in Cy_1. Residual activity on the other two kinases was found but SAR was still clearly observable.

The derivatives **52**–**54** which represent the **IId** family (entries 18–20) were inactive on DYRK1A; their best effect was found against CDK5 in the submicromolar range. In addition, the activity of **35** and **54** on GSK3 remained unchanged whereas the activity values on DYRK1A/CDK5 were reversed. A novel

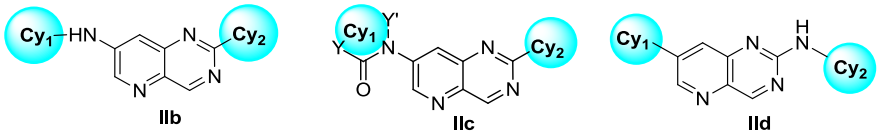


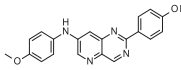
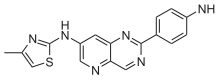
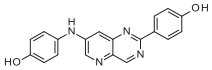
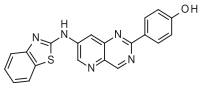
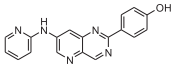
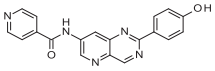
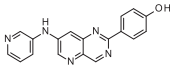
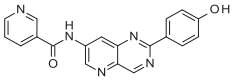
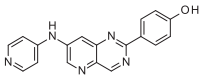
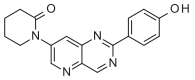
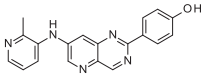
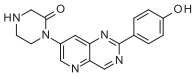
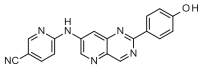
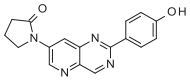
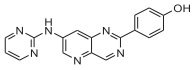
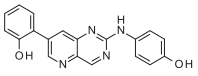
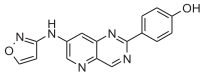
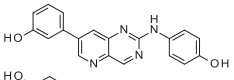
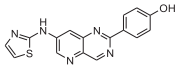
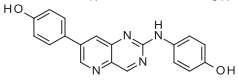
Scheme 2. Preparation of thiophene containing compounds **31**-**33**. *Reagents and conditions* : a) secondary amine (5.0 eq.), NaBH(OAc)3 (2.0 eq.), DME, 60°C, 5 h ; b) crude boronic acids **28**-**30** (5.0 eq.), K2CO3 (2.0 eq.), Pd(PPh3)4 (5 mol %), toluene/EtOH 2/1, 150°C, mW, 10 min, **31** 55%, **32** 50% or **33** 52%.



Scheme 3. Preparation of *C*-2 or *C*-7 amino/amido derivatives. *Reagents and conditions* : a) (Het)ArNH2 (1.2 eq.), Pd(OAc)2 (10 mol %), xantphos (20 mol %), K2CO3 (2.0 eq.), dioxane, 140°C, mW, 60 min, **34**-**45** 57-97 % ; b) idem with amides (1.2 eq.), Pd(OAc)2 (5 mol %), xantphos (5 mol %), 70 min, **46**-**50** 78-93%, c) $4\text{-}HOC_6H4NH2$ (1.05 eq.), dioxane, rflx, 24 h, 60% ; d) hydroxyphenyl boronic acids (1.2 eq.), K2CO3 (2.0 eq.), Pd(PPh3)4 (5 mol %), toluene/EtOH 2/1, 150°C, mW, 10 min, **52** R = 2-OH 66%, **53** R = 3-OH 60% and **54** R = 4-OH 80%.

Table 2
Full report of the kinase inhibition of the **IIb** and **IIc** library.



Entry	Compound (yield)	Kinase inhibition IC_{50}, μM[a]			Entry	Compound (yield)	Kinase inhibition IC_{50}, μM[a]		
		DYRK 1A	CDK5	GSK3			DYRK 1A	CDK5	GSK3
1	 **34** (57%)	>10	1.8	7.3	11	 **44** (67%)	>10	>10	>10
2	 **35** (80%)	0.8	1.4	5.2	12	 **45** (87%)	>10	5.3	>10
3	 **36** (90%)	>10	5	>10	13	 **46** (89%)	4.2	>10	>10
4	 **37** (71%)	>10	>10	>10	14	 **47** (88%)	>10	>10	>10
5	 **38** (72%)	>10	>10	>10	15	 **48** (79%)	**0.06**	2.2	4.6
6	 **39** (97%)	>10	>10	>10	16	 **49** (78%)	5.1	>10	>10
7	 **40** (80%)	>10	4	5.5	17	 **50** (93%)	1	>10	>10
8	 **41** (81%)	>10	7.6	>10	18	 **52** (66%)	>10	3.7	>10
9	 **42** (52%)	2	2.1	>10	19	 **53** (60%)	>10	0.71	4.5
10	 **43** (84%)	>10	2.2	>10	20	 **54** (80%)	>10	0.84	7.1

[a] Values are means of three measured values of IC_{50}.

orientation of the compound in the active site due to the 4-hydroxyaniline moiety may explain the biological results, as suggested by docking studies.

The most attractive result of this nitrogen incorporation is indubitably the fusion of the amide link and Cy_1. The activities of derivatives **48**–**50** were fully restored on DYRK1A and a great selectivity was observed, despite values in the micromolar range. The most active molecule on DYRK1A was **48** which had an IC_{50} of 60 nM and a 27- and 76- fold selectivity towards CDK5 or GSK3, respectively. Interestingly, this compound has the highest ligand efficiency (LE = 0.42) and lipophilic efficiency (LiPE = 4.29) amongst all the synthesized compounds.

4. Molecular modelling

Docking experiments revealed that the binding mode of most of the compounds is conserved in the two protein kinases DYRK1A and CDK5, characteristic of the binding mode of typical type I kinase inhibitors [9]. The key interactions between the hetero-aromatic scaffold **IIa** and the hinge region of the protein kinases take place through hydrogen bond networks. The hydrogen acceptor atom *N-4* of scaffold **IIa** interacts with the backbone NH of Leu241 of DYRK1A. The weak acidic hydrogen on position 5 of scaffold **IIa** interacts with oxygen of the backbone carbonyl of Glu239 and Cys83 of DYRK1A and CDK5 respectively. The Cy_1 substituent points towards the solvent area while Cy_2 binds to the hydrophobic pocket close to the gatekeeper residue [10]. The hydroxyl group of Cy_2 hydroxyphenyl found in many compounds is involved in a hydrogen bond network with catalytic Lys188 of DYRK1A or Asp307 from the DFG motif (Fig. 2).

The binding mode of compound **48**, the most efficient ligand, is shown in Fig. 3. The hydroxyl group of Cy_2 creates a hydrogen bond with Lys188 and the oxo-piperidine points to the solvent area. Interestingly, docking results suggest that the scaffold **IId** is flipped 180° in the active site compared to scaffolds **IIa**, **IIb**, and **IIc** when Cy_2 is a hydroxyaniline moiety (compounds **52**–**54**).

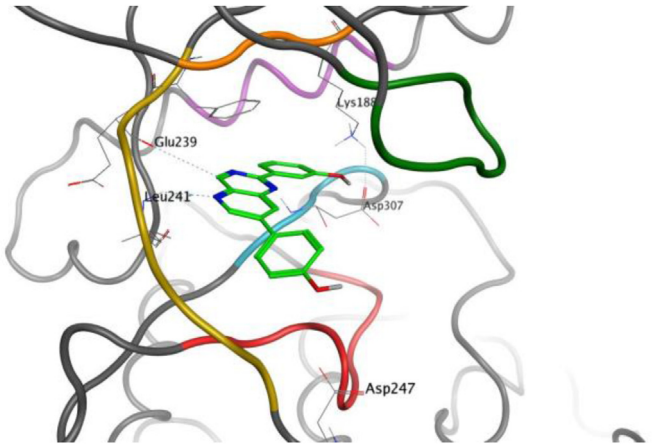


Fig. 2. Binding mode of compound **4** in DYRK1A active site. Analysis of the hydrogen bonding interactions and hydrophobic contacts made by compound **4** (green) to residues in the DYRK1A ATP binding pocket. The three-letter amino acid code and residue number are labeled next to each side chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4 shows compound **53** bound to CDK5. Here, the scaffold in the CDK5 active site is better oriented compared to the other scaffolds **IIb** and **IIc**. The hydrogen acceptor atom *N-4* of scaffold **IId** interacts with the backbone NH of Cys83 of CDK5 and the Cy_1 hydroxyphenyl forms a hydrogen bond with Asp144. The good selectivity observed towards GSK3β can be attributed to a proline residue (Pro136) located in the hinge region, preventing backbone NH from creating a hydrogen bond with the scaffold. This major difference between the three protein kinases could explain the overall poor GSK3β biological activities of the three scaffolds compared to DYRK1A and CDK5.

5. Conclusion

In this paper, we have presented the design and synthesis of novel 4-7-disubstituted pyrido[3,2-*d*]pyrimidines scaffolds adopting a peculiar V-shaped conformation. The collection was generated from a single starting material, the 4,7-dichloropyrido[3,2-*d*] pyrimidine which afford the final compounds after two steps. We have elaborated the most straightforward routes to access to final

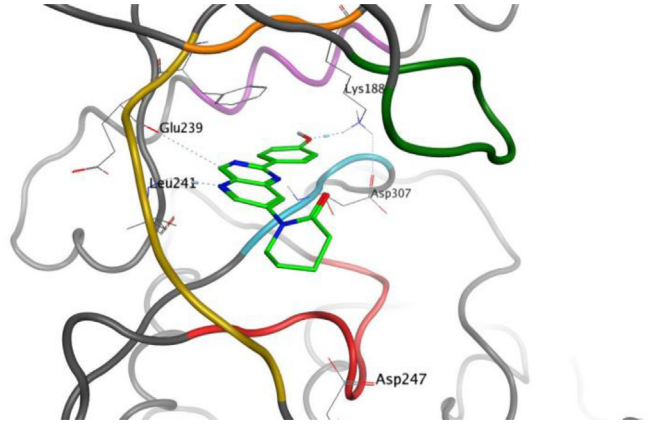


Fig. 3. Binding mode of compound **48** in DYRK1A active site. Analysis of the hydrogen bonding interactions and hydrophobic contacts made by compound **48** (green) to residues in the DYRK1A ATP binding pocket. The three-letter amino acid code and residue number are labeled next to each side chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

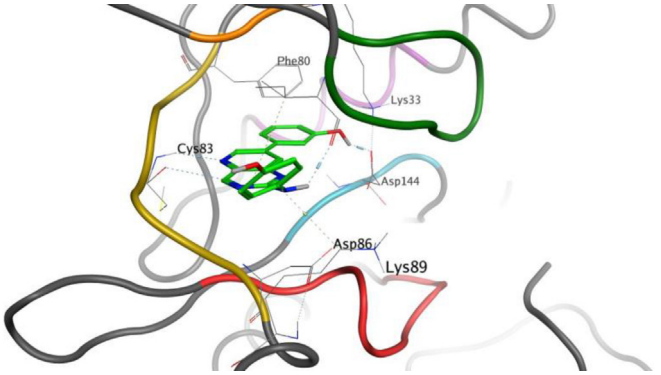


Fig. 4. Binding mode of compound **53** in CDK5 active site. Analysis of the hydrogen bonding interactions and hydrophobic contacts made by compound **53** (green) to residues in the CDK5 ATP binding pocket. The three-letter amino acid code and residue number are labeled next to each side chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

derivatives. Sequential or one-pot sequence including selective cross coupling reactions in *C*-4 followed by the second one in *C*-7 are also achieved with success. In position *C*-4, a Suzuki-Myaura type reaction led to monosubstituted derivatives whereas in *C*-7 a Suzuki or a Buchwald type reaction achieves the synthesis using commercially available or unknown boron derivatives. These drug-like molecules have been tailored as dual selective DYRK1A and CDK5 kinase inhibitors with an excellent ligand efficiency of 0.4. A counter-screen on GSK3, a protein kinase belonging to the same CMGC group, confirms the selectivity profile of these compounds. Molecular modelling was used to propose specific substituents for activity and selectivity. The proposed binding mode of the compounds is in good agreement with the structure–activity relationship. These molecules are promising scaffolds for targeting protein kinases involved in the central nervous system.

6. Experimental section

6.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 250 MHz or 400 MHz instrument using $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in parts per million (δ scale) and all coupling constant (J) values are in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet doublet). Melting points are uncorrected. IR absorption spectra were obtained on a Perkin Elmer PARAGON 1000 PC and values are reported in cm^{-1}. HRMS were recorded on a Maxis Bruker instrument. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F_{254}). Spots were visualized by UV light at 254 nm and 356 nm. Column chromatographies were performed using silica gel 60 (0.063–0.200 mm, Merck). Microwave experiments were performed on a Biotage Initiator apparatus.

6.1.1. *4-(7-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-2-yl) thiophene-2-carbalde-hyde* ***23***

In an argon degassed solution, 2,7-dichloropyrido[3,2-*d*]pyrimidine **22** (200 mg, 0.99 mmol) was dissolved at room temperature in a toluene/EtOH 2/1 mixture. 2-Formyl-4-thiophene boronic acid (155 mg, 0.99 mmol, 1.0 eq.), Na_2CO_3, (210.0 mg, 1.98 mmol, 2.0 eq.) and finally the catalyst $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 0.05 eq.) were successively added prior to placing the flask in a preheated oil bath at 100 °C. After 6 h of stirring, the reaction mixture was cooled at room temperature and the 4-

hydroxyphenyl boronic acid (164.0 mg, 1.18 mmol, 1.2 eq.) was added with a small additional amount of $Pd(PPh_3)_4$ (1.1 mg, 0.05 mmol, 0.01 e). The reaction mixture was heated at 100 °C for one additional hour and cooled at room temperature. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography on silica gel (CH_2Cl_2/Et_3N 99/1 then CH_2Cl_2/MeOH 98/2) to afford **23** as a pale brown solid in 24% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, cm^{-1}) ν 3389, 1696, 1584, 1472, 1228, 1174, 834, 725; ^1H NMR (400 MHz, DMSO-d_6) δ 6.95 (d, 2H, J = 8.6 Hz, H_{Ph}), 7.86 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.49 (d, 1H, J = 1.8 Hz, H_8), 8.76 (d, 1H, J = 1.2 Hz, H_{Th}), 9.00 (s, 1H, H_{Th}), 9.40 (d, 1H, J = 1.8 Hz, H_6), 9.65 (s, 1H, H_4), 9.97 (br, 1H, OH), 10.07 (d, 1H, J = 1.2 Hz, CHO); ^{13}C DEPT NMR (100.6 MHz, DMSO-d_6) δ 116 (2xCH), 129 (2xCH), 130 (CH), 137 (CH), 137 (CH), 151 (CH), 161 (CH), 184 (CHO); HRMS (EI-MS): calculated for $C_{18}H_{12}N_3O_2S$ 334.06447 $[M+H]^+$ found 344.06435 $[M+H]^+$.

6.1.2. 7-(4-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-2-yl) thiophene-3-carbalde-hyde **25**

Under argon, in a sealed vial, compound **24** (91 mg, 0.35 mmol) was dissolved at room temperature in a mixture of toluene/EtOH 2/1 and 2-formyl-4-thiophene boronic acid (66 mg, 0.42 mmol, 1.2 eq.), K_2CO_3 (98 mg, 0.70 mmol, 2.0 eq.) and $Pd(PPh_3)_4$ (21 mg, 0.017 mmol, 0.05 eq.) were successively added. The mixture was heated under microwave irradiation at 150 °C for 10 min. After cooling, the volatiles were removed under pressure and the crude material was purified by flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) to afford **25** as a brown solid in 74% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3354, 1696, 1569, 1441, 1253, 1139, 845, 697; ^1H NMR (400 MHz, DMSO-d_6) δ 6.93 (d, 2H, J = 8.5 Hz, H_{Ph}), 8.41 (d, 2H, J = 8.5 Hz, H_{Ph}), 8.75 (s, 1H, H_{Th}), 8.86 (s, 1H, H_8), 9.00 (s, 1H, H_6), 9.49 (s, 1H, H_{Th}), 9.63 (s, 1H, H_4), 10.02 (s, 1H, OH), 10.10 (s, 1H, CHO); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 115 (2 × CH), 127 (Cq), 130 (2 × CH), 130 (CH), 134 (Cq), 134 (CH), 136 (CH), 137 (Cq), 138 (Cq), 144 (Cq), 146 (Cq), 150 (CH), 160 (Cq), 160 (Cq), 161 (CH), 184 (CHO); HRMS (EI-MS): calculated for $C_{18}H_{12}N_3O_2S$ 334.06500 $[M+H]^+$ found 344.06400 $[M+H]^+$.

6.1.3. 4-(7-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-2-yl) thiophene-3-carbaldehyde **26**

Compound **26** was obtained from **24** as described for compound **25** using 3-formyl-4-thiopheneboronic acid. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) afforded the attempted product as a dark brown solid in 68% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 240–242 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3087, 1701, 1571, 1438, 1226, 1160, 827, 737; ^1H NMR (400 MHz, DMSO-d_6) δ 6.93 (d, 2H, J = 8.5 Hz, H_{Ph}), 8.09 (d, 1H, J = 2.7 Hz, H_{Th}), 8.40–8.43 (m, 3H, H_{Ph} and H_{Th}), 8.82 (s, 1H, J = 2.0 Hz, H_8), 9.06 (s, 1H, J = 2.0 Hz, H_6), 9.65 (s, 1H, H_4), 9.98 (s, 1H, CHO), 10.09 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 115 (2 × CH), 127 (Cq), 130 (CH), 130 (2xCH), 134 (CH), 135 (Cq), 136 (Cq), 137 (Cq), 139 (Cq), 142 (CH), 146 (Cq), 152 (CH), 160 (Cq), 160 (Cq), 161 (CH), 186 (CHO); HRMS (EI-MS): calculated for $C_{18}H_{12}N_3O_2S$ 334.06500 $[M+H]^+$ found 344.06660 $[M+H]^+$.

6.1.4. 4-[7-(5-Methyl-thiophen-3-yl)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **27**

Compound **25** (203.0 mg, 0.60 mmol) was dissolved in a mixture MeOH/DMF 1/1 and the resulting solution was stirred for 5 min. The temperature was lowered to −10 °C then $NaBH_4$ (0.3 mmol, 0.5 eq.) was added and the reaction mixture was stirred at room temperature overnight. Volatiles were removed under reduced pressure and the crude material was purified by flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 95/5) to afford **27** as a white solid in 71% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3353, 1578, 1452, 1236, 1148, 840, 739; ^1H NMR (400 MHz, DMSO-d_6) δ 4.73 (s, 2H, CH_2), 5.64 (s, 1H, OH), 6.93 (d, 2H, J = 8.4 Hz, H_{Ph}), 7.76 (s, 1H, H_{Th}), 8.36 (s, 1H, H_{Th}), 8.40 (d, 2H, J = 8.4 Hz, H_{Ph}), 8.58 (s, 1H, H_8), 9.44 (s, 1H, H_6), 9.59 (s, 1H, H_4), 10.10 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 58 (CH_2), 115 (2xCH), 122 (CH), 124 (CH), 127 (Cq), 129 (CH), 130 (2xCH), 135 (Cq), 136 (Cq), 137 (Cq), 146 (Cq), 148 (Cq), 150 (CH), 160 (Cq), 160 (Cq), 161 (CH); HRMS (EI-MS) : calculated for $C_{18}H_{14}N_3O_2S$ m/z 336.08012 $[M+H]^+$ found 336.08049 $[M+H]^+$.

6.1.5. General procedure for the synthesis of crude boronic acids **28**–**30**

2-Formyl-4-thiophene boronic acid (100 mg, 0.64 mmol) was dissolved in DME (10 mL) and the appropriate amine (3.2 mmol, 5.0 eq.) was added, followed by a drop of AcOH. The resulting solution was stirred for 5 min at room temperature. $NaBH(OAc)_3$ (271 mg, 2.0 eq.) was added and the resulting solution was heated at 60 °C for 5 h. The volatiles were evaporated under reduced pressure and the crude material was used without further purification in the next step.

6.1.6. 4-(7-(5-(Piperidin-1-ylmethyl)thiophen-3-yl)pyrido[3,2-d] pyrimidin-2-yl)phenol **31**

Compound **31** was obtained from **24** as described for compound **25** using boronic acid **28**. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/THF 9/1) afforded the attempted product as a yellowish solid in 55% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3334, 1577, 1451, 1225, 1158, 847, 703; ^1H NMR (400 MHz, DMSO-d_6) δ 1.39 (s, 2H, H_{PP}), 1.51 (s, 4H, H_{PP}), 2.42 (s, 4H, H_{PP}), 3.71 (s, 2H, CH_2), 6.92 (d, 2H, J = 8.7 Hz, H_{Ph}), 7.75 (s, 1H, H_{Th}), 8.34 (d, 1H, J = 1.2 Hz, H_{Th}), 8.40 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.57 (d, 1H, J = 1.7 Hz, H_8), 9.42 (d, 1H, J = 1.7 Hz, H_6), 9.57 (s, 1H, H_4), 10.12 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 23 (CH_2), 25 (2 × CH_2), 53 (2 × CH_2), 57 (CH_2), 115 (2 × CH), 124 (CH), 125 (CH), 127 (Cq), 129 (CH), 130 (2 × CH), 135 (Cq), 136 (Cq), 137 (Cq), 144 (Cq), 146 (Cq), 150 (CH), 160 (Cq), 160 (Cq), 161 (CH); HRMS (EI-MS) : calculated for $C_{23}H_{23}N_4OS$ 403.15871 $[M+H]^+$ found 403.15892 $[M+H]^+$.

6.1.7. 4-(7-(5-(Morpholinomethyl)thiophen-3-yl)pyrido[3,2-d] pyrimidin-2-yl)phenol **32**

Compound **32** was obtained from **24** as described for compound **25** using boronic acid **29**. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/THF 9/1) afforded the attempted product as a brown solid in 50% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3400, 1571, 1443, 1220, 1153, 803, 706; ^1H NMR (400 MHz, DMSO-d_6) δ 2.45 (t, 4H, J = 4.3 Hz, H_m), 3.60 (t, 4H, J = 4.3 Hz, H_m), 3.74 (s, 2H, CH_2), 6.92 (d, 2H, J = 8.7 Hz, H_{Ph}), 7.77 (s, 1H, H_{Th}), 8.36 (d, 1H, J = 0.9 Hz, H_{Th}), 8.40 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.56 (d, 1H, J = 1.6 Hz, H_8), 9.41 (d, 1H, J = 1.6 Hz, H_6), 9.57 (s, 1H, H_4), 10.07 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 52 (2 × CH_2), 56 (CH_2), 66 (2 × CH_2), 115 (2 × CH), 125 (CH), 125 (CH), 127 (Cq), 129 (CH), 130 (2 × CH), 135 (Cq), 136 (Cq), 137 (Cq), 143 (Cq), 146 (Cq), 150 (CH), 160 (Cq), 160 (Cq), 160 (CH); HRMS (EI-MS): calculated for $C_{22}H_{21}N_4O_2S$ 405.13797 $[M+H]^+$ found 405.13833 $[M+H]^+$.

6.1.8. 4-(7-(5-(4-Methylpiperazin-1-ylmethylthiophen-3-yl)pyrido [3,2-d]pyrimidin-2-yl)phenol **33**

Compound **33** was obtained from **24** as described for compound **25** using boronic acid **30**. Flash chromatography using silica gel ($AcOEt/NH_3$ 99/1 then AcOEt/MeOH 95/5) afforded the attempted product as a yellow solid in 52% yield. R_f: 0.2 (petroleum ether/

EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3387, 1571, 1462, 1234, 1152, 844, 740; ^1H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 2.37 (br s, 8H, H_{Pr}), 3.74 (s, 2H, CH_2), 6.93 (d, 2H, $J = 8.7$ Hz, H_{Ph}), 7.78 (d, 1H, $J = 0.9$ Hz, H_{Th}), 8.37 (d, 1H, $J = 0.9$ Hz, H_{Th}), 8.41 (d, 2H, $J = 8.7$ Hz, H_{Ph}), 8.59 (d, 1H, $J = 1.9$ Hz, H_8), 9.43 (d, 1H, $J = 1.9$ Hz, H_6), 9.59 (s, 1H, H_4), 10.09 (br s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 46 (CH_3), 52 (2 × CH_2), 55 (2 × CH_2), 56 (CH_2), 116 (2 × CH), 125 (CH), 125 (CH), 128 (Cq), 130 (CH), 130 (2 × CH), 135 (Cq), 136 (Cq), 137 (Cq), 144 (Cq), 147 (Cq), 150 (CH), 160 (Cq), 161 (Cq), 161 (CH); HRMS (EI-MS): calculated for $C_{23}H_{24}N_5OS$ 418.16961 $[M+H]^+$ found 418.16992 $[M+H]^+$.

6.1.9. General procedure A for preparation of compounds IIb–c

In a degassed solution of dioxane containing **24** (1.0 mmol), primary amines (for **IIb**) or amides for **IIc** (1.2 eq.), K_2CO_3 (2.0 eq.) and xantphos (0.2 eq; for **IIb** or 0.1 eq. for **IIc**) was added $Pd(OAc)_2$ (0.1 eq; for **IIb** or 0.05 eq. for **IIc**) and the suspension immediately placed in the microwave cavity. After irradiation (60 min for **IIb** or 70 min for **IIc**) at 140 °C, the solution was cooled and volatiles were removed under reduced pressure and the crude material was purified by recrystallization or by flash chromatography on silica gel.

6.1.10. 4-[7-(4-Methoxy-phenylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **34**

Compound **34** was obtained following the general procedure **A** using paramethoxyaniline which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a reddish solid in a 57% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 134–136 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3298, 1573, 1449, 1233, 1159, 830, 702; ^1H NMR (400 MHz, DMSO-d_6) δ 3.78 (s, 3H, CH_3), 6.88 (d, 2H, $J = 8.2$ Hz, H_{Ph}), 7.01 (d, 2H, $J = 8.4$ Hz, H_{Ph}), 7.22 (s, 1H, H_8), 7.28 (d, 2H, $J = 8.4$ Hz, H_{Ph}), 8.32 (d, 2H, $J = 8.2$ Hz, H_{Ph}), 8.64 (s, 1H, H_6), 9.18 (s, 1H, H_4), 9.21 (s, 1H, NH), 9.97 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 55 (CH_3), 106 (CH), 114 (2 × CH), 115 (2 × CH), 123 (2 × CH), 128 (Cq), 129 (2xCH), 132 (Cq), 132 (Cq), 145 (CH), 145 (Cq), 148 (Cq), 155 (Cq), 158 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{20}H_{17}N_4O_2$ 345.13520 $[M+H]^+$ found 345.13600 $[M+H]^+$.

6.1.11. 4-[7-(4-Hydroxyphenylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **35**

Compound **35** was obtained following the general procedure **A** using parahydroxyaniline which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/02) as a light brown solid in an 80% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 272 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3415, 1581, 1434, 1222, 1158, 835, 701; ^1H NMR (400 MHz, DMSO-d_6) δ 6.85–6.89 (m, 4H, H_{Ph}), 7.15 (d, 1H, $J = 2.4$ Hz, H_8), 7.17 (d, 2H, $J = 8.6$ Hz, H_{Ph}), 8.32 (d, 2H, $J = 8.7$ Hz, H_{Ph}), 8.62 (d, 1H, $J = 2.4$ Hz, H_6), 9.07 (s, 1H, H_4), 9.20 (s, 1H, NH), 9.45 (s, 1H, OH), 9.95 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 106 (CH), 115 (2xCH), 116 (2 × CH), 123 (2 × CH), 128 (Cq), 130 (2 × CH), 130 (Cq), 132 (Cq), 145 (CH), 146 (Cq), 148 (Cq), 154 (Cq), 158 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{19}H_{15}N_4O_2$ 331.11950 $[M+H]^+$ found 331.11830 $[M+H]^+$.

6.1.12. 4-[7-(Pyridin-2-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **36**

Compound **36** was obtained following the general procedure **A** using 2-aminopyridine which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH3 99/1 then CH_2Cl_2/MeOH 98/2) as a reddish solid in a 90% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3452, 1576, 1453, 1280, 1166, 805, 703; ^1H NMR (400 MHz, DMSO-d_6) δ 6.90 (d, 2H, $J = 8.5$ Hz, H_{Ph}), 6.97 (dd, 1H, $J = 5.4$, 6.6 Hz, H_P), 7.05 (d, 1H, $J = 8.3$ Hz, H_P), 7.71–7.78 (m, 1H, H_P), 8.38 (d, 3H, $J = 8.5$ Hz, $H_{Ph\ and\ P}$), 8.90 (d, 1H, $J = 2.0$ Hz, H_8), 9.04 (d, 1H, $J = 2.0$ Hz, H_6), 9.33 (s, 1H, H_4), 9.99 (s, 1H, NH), 10.13 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 112 (CH), 114 (CH), 115 (2 × CH), 116 (CH), 128 (Cq), 130 (2 × CH), 132 (Cq), 137 (CH), 141 (Cq), 145 (CH), 147 (CH), 148 (Cq), 154 (Cq), 159 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{18}H_{14}N_5O$ 316.11980 $[M+H]^+$ found 316.12100 $[M+H]^+$.

6.1.13. 4-[7-(Pyridin-3-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **37**

Compound **37** was obtained following the general procedure **A** using 3-aminopyridine which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a yellowish solid in a 71% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3456, 1556, 1451, 1243, 1158, 845, 696; ^1H NMR (400 MHz, DMSO-d_6) δ 6.87 (d, 2H, $J = 8.6$ Hz, H_{Ph}), 7.42 (dd, 1H, $J = 4.7$, 8.1 Hz, H_P), 7.51 (s, 1H, H_P), 7.84 (d, 1H, $J = 8.1$ Hz, H_P), 8.34 (d, 3H, $J = 8.6$ Hz, $H_{Ph\ and\ P}$), 8.59 (s, 1H, H_8), 8.73 (s, 1H, H_6), 9.29 (s, 1H, H_4), 9.55 (s, 1H, NH), 10.00 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 109 (CH), 115 (2 × CH), 124 (CH), 126 (CH), 128 (Cq), 130 (2 × CH), 132 (Cq), 136 (Cq), 142 (CH), 144 (CH), 144 (Cq), 145 (CH), 148 (Cq), 158 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{18}H_{14}N_5O$ 316.11980 $[M+H]^+$ found 316.11890 $[M+H]^+$.

6.1.14. 4-[7-(Pyridin-4-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **38**

Compound **38** was obtained following the general procedure **A** using 4-aminopyridine which afforded the attempted derivative after a recrystallization in methanol as a dark brown solid in a 72% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3414, 1572, 1453, 1290, 1156, 810, 703; ^1H NMR (400 MHz, DMSO-d_6) δ 6.89 (d, 2H, $J = 8.7$ Hz, H_{Ph}), 7.29 (d, 2H, $J = 6.2$ Hz, H_P), 7.86 (d, 1H, $J = 2.4$ Hz, H_8), 7.94 (d, 1H, $J = 4.9$ Hz, NH), 8.38 (d, 2H, $J = 8.7$ Hz, H_{Ph}), 8.43 (d, 2H, $J = 6.2$ Hz, H_P), 8.80 (d, 1H, $J = 2.4$ Hz, H_6), 9.39 (s, 1H, H_4), 9.84 (br s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 111 (2 × CH), 113 (CH), 115 (2 × CH), 127 (Cq), 130 (2xCH), 133 (Cq), 141 (Cq), 146 (CH), 147 (Cq), 147 (Cq), 149 (Cq), 150 (2 × CH), 159 (CH), 160 (Cq); HRMS (EI-MS): calculated for $C_{18}H_{14}N_5O$ 316.11980 $[M+H]^+$ found 316.11990 $[M+H]^+$.

6.1.15. 4-[7-(2-(Methyl)pyridinylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **39**

Compound **39** was obtained following the general procedure **A** using 3-amino-2-methylpyridine which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a brown solid in a 97% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 186–188 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3404, 1577, 1459, 1274, 1156, 843, 751; ^1H NMR (400 MHz, DMSO-d_6) δ 2.38 (s, 3H, CH_3), 6.90 (d, 2H, $J = 8.6$ Hz, H_{Ph}), 6.95 (dd, 1H, $J = 4.4$, 7.1 Hz, H_P), 7.59 (d, 1H, $J = 7.1$ Hz, H_P), 8.23 (d, 1H, $J = 4.4$ Hz, H_P), 8.38 (d, 2H, $J = 8.6$ Hz, H_{Ph}), 8.82 (s, 1H, NH), 8.85 (d, 1H, $J = 2.1$ Hz, H_8), 9.16 (d, 1H, $J = 2.1$ Hz, H_6), 9.34 (s, 1H, H_4), 10.00 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 17.1 (CH_3), 115 (2 × CH), 115 (CH), 117 (CH), 120 (Cq), 128 (Cq), 130 (2 × CH), 133 (Cq), 138 (CH), 142 (Cq), 144 (CH), 146 (CH), 147 (Cq), 152(Cq), 195 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{19}H_{16}N_5O$ 330.13550 $[M+H]^+$ found 330.13470 $[M+H]^+$.

6.1.16. 4-[7-(5-(Cyano)pyridinylamino]-pyrido[3,2-d]pyrimidin-2-yl]-phenol **40**

Compound **40** was obtained following the general procedure **A** using 2-amino-5-cyano-pyridine which afforded the attempted

derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/MeOH 95/5) as a reddish solid in an 80% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3311, 2224, 1573, 1433, 1280, 1153, 828, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.89 (d, 2H, J = 8.6 Hz, H_{Ph}), 7.07 (d, 1H, J = 8.7 Hz, H_P), 8.03 (dd, 1H, J = 2.0, 8.7 Hz, H_P), 8.35 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.77 (d, 1H, J = 1.6 Hz, H_8), 8.86 (d, 1H, J = 2.0 Hz, H_P), 8.92 (d, 1H, J = 1.6 Hz, H_6), 9.34 (s, 1H, H_4), 10.01 (s, 1H, OH), 10.59 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 100 (Cq), 112 (CH), 115 (2 × CH), 117 (CH), 117 (Cq), 128 (Cq), 130 (2 × CH), 133 (Cq), 140 (CH), 140 (Cq), 145 (CH), 147 (Cq), 152 (CH), 156 (Cq), 159 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{19}H_{13}N_6O$ 341.11510 $[M+H]^+$ found 341.11540 $[M+H]^+$.

6.1.17. 4-[7-(Pyrimidin-2-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **41**

Compound **41** was obtained following the general procedure **A** using 2-aminopyrimidine which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/NH_3 98/2) as a reddish solid in an 81% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3405, 1574, 1437, 1266, 1157, 803, 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (d, 2H, J = 8.4 Hz, H_{Ph}), 7.08 (t, 1H, J = 4.6 Hz, H_{Pm}), 8.38 (d, 2H, J = 8.4 Hz, H_{Ph}), 8.68 (d, 2H, J = 4.6 Hz, H_{Pm}), 8.96 (s, 1H, H_8), 9.09 (s, 1H, H_6), 9.38 (s, 1H, H_4), 10.01 (s, 1H, OH), 10.64 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 114 (CH), 115 (2 × CH), 116 (CH), 128 (Cq), 130 (2xCH), 133 (Cq), 140 (Cq), 145 (CH), 147 (Cq), 158 (2 × CH), 159 (Cq), 159 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{17}H_{13}N_6O$ 317.11510 $[M+H]^+$ found 317.11420 $[M+H]^+$.

6.1.18. 4-[7-(Isoxazol-3-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **42**

Compound **42** was obtained following the general procedure **A** using 3-amino-1,2-oxazole which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a yellow solid in a 52% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3400, 1560, 1459, 1272, 1150, 805, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.43 (s, 1H, H_{iz}), 6.89 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.39 (d, 3H, J = 8.6 Hz, $H_{Ph\ and}$ H_{iz}), 8.80–8.83 (m, 2H, H_8 and H_6), 9.39 (s, 1H, H_4), 10.01 (s, 1H, OH), 10.41 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 98 (CH), 114 (CH), 115 (2 × CH), 128 (Cq), 130 (2 × CH), 132 (Cq), 141 (Cq), 144 (CH), 148 (Cq), 159 (CH), 159 (Cq), 159 (CH), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{16}H_{12}N_5O_2$ 306.09910 $[M+H]^+$ found 306.09820 $[M+H]^+$.

6.1.19. 4-[7-(Thiazol-2-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **43**

Compound **43** was obtained following the general procedure **A** using 2-amino-1,3-thiazole which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a green solid in an 84% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3412, 1566, 1443, 1247, 1122, 840, 697; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.90 (d, 2H, J = 8.7 Hz, H_{Ph}), 7.17 (d, 1H, J = 3.6 Hz, H_{Tz}), 7.49 (d, 1H, J = 3.6 Hz, H_{Tz}), 8.38 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.84 (d, 1H, J = 2.0 Hz, H_8), 8.91 (d, 1H, J = 2.0 Hz, H_6), 9.73 (s, 1H, H_4), 10.04 (s, 1H, OH), 11.35 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 111 (CH), 114 (CH), 115 (2 × CH), 128 (Cq), 130 (2 × CH), 133 (Cq), 139 (CH), 141 (Cq), 144 (CH), 148 (Cq), 159 (CH), 160 (Cq), 160 (Cq), 162 (Cq); HRMS (EI-MS): calculated for $C_{16}H_{12}N_5OS$ 322.07630 $[M+H]^+$ found 322.07730 $[M+H]^+$.

6.1.20. 4-[7-(4-Methyl-thiazol-2-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **44**

Compound **44** was obtained following the general procedure **A** using 2-amino-4-methyl-1,3-thiazole which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/02) as a green solid in a 67% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3465, 1568, 1452, 1201, 1166, 806, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.36 (s, 3H, CH_3), 6.73 (s, 1H, H_{Tz}), 6.90 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.38 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.80 (d, 1H, J = 1.9 Hz, H_8), 8.86 (d, 1H, J = 1.9 Hz, H_6), 9.37 (s, 1H, H_4), 10.01 (s, 1H, OH), 11.16 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 17 (CH_3), 105 (CH), 114 (CH), 115 (2xCH), 128 (Cq), 130 (2xCH), 133 (Cq), 141 (Cq), 145 (CH), 148 (Cq), 149 (Cq), 159 (CH), 160 (Cq), 161 (Cq), 161 (Cq); HRMS (EI-MS): calculated for $C_{17}H_{14}N_5OS$ 336.09136 $[M+H]^+$ found 336.09130 $[M+H]^+$.

6.1.21. 4-[7-(Benzothiazol-2-ylamino)-pyrido[3,2-d]pyrimidin-2-yl]-phenol **45**

Compound **45** was obtained following the general procedure **A** using 2-aminobenzothioazole which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH3 99/1 then CH_2Cl_2/MeOH 98/2) as a dark green solid in an 87% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3408, 1569, 1443, 1269, 1159, 845, 722; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (d, 2H, J = 8.4 Hz, H_{Ph}), 7.28 (t, 1H, J = 7.5 Hz, H_{bT}), 7.44 (t, 1H, J = 7.5 Hz, H_{bT}), 7.84 (d, 1H, J = 7.9 Hz, H_{bT}), 7.92 (d, 1H, J = 7.9 Hz, H_{bT}), 8.41 (d, 2H, J = 8.4 Hz, H_{Ph}), 8.91 (d, 1H, J = 2.0 Hz, H_8), 9.11 (d, 1H, J = 2.0 Hz, H_6), 9.40 (s, 1H, H_4), 10.05 (br s, 1H, OH), 11.46 (br s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 115 (2 × CH), 116 (CH), 120 (CH), 121 (CH), 123 (CH), 126 (CH), 128 (Cq), 130 (2 × CH), 130 (Cq), 133 (Cq), 140 (Cq), 144 (CH), 147 (Cq), 151 (Cq), 159 (CH), 160 (Cq), 160 (Cq), 160 (Cq); HRMS (EI-MS): calculated for $C_{20}H_{14}N_5OS$ 372.09136 $[M+H]^+$ found 372.09136 $[M+H]^+$.

6.1.22. N-[2-(4-Hydroxyphenyl)-pyrido[3,2-d]pyrimidin-7-yl] isonicotinamide **46**

Compound **46** was obtained following the general procedure **A** using isonicotinamide, which afforded the attempted derivative after a purification under flash chromatography on silica gel CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 95/05) as a yellowish solid in an 89% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 230–232 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3406, 1671, 1558, 1447, 1264, 1156, 846, 697; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (d, 2H, J = 8.6 Hz, H_{Ph}), 7.94 (d, 2H, J = 5.6 Hz, H_P), 8.41 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.85–8.86 (m, 3H, H_P and H_8), 9.21 (d, 1H, J = 1.9 Hz, H_6), 9.53 (s, 1H, H_4), 10.06 (s, 1H, OH), 11.25 (s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 115 (2 × CH), 121 (CH), 121 (2 × CH), 127 (Cq), 130 (2xCH), 135 (Cq), 139 (Cq), 140 (Cq), 146 (CH), 147 (Cq), 150 (2xCH), 160 (Cq), 160 (CH), 160 (Cq), 165 (Cq); HRMS (EI-MS): calculated for $C_{19}H_{14}N_5O_2$ 344.11470 $[M+H]^+$ found 344.11460 $[M+H]^+$.

6.1.23. N-[2-(4-Hydroxyphenyl)-pyrido[3,2-d]pyrimidin-7-yl] nicotinamide **47**

Compound **47** was obtained following the general procedure **A** using nicotinamide, which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 95/05) as a yellowish solid in an 88% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 226–228 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3444, 1672, 1553, 1448, 1230, 1157, 807, 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.93 (d, 2H, J = 8.6 Hz, H_{Ph}), 7.62 (dd, 1H, J = 4.8, 7.7 Hz, H_P), 8.41 (d, 3H, J = 8.6 Hz, H_{Ph} and H_P), 8.83 (d, 1H, J = 4.8 Hz, H_P), 8.88 (d, 1H, J = 2.0 Hz, H_8), 9.23 (s, 1H, H_P), 9.26 (d, 1H, J = 2.0 Hz, H_6), 9.52 (s, 1H, H_4), 10.10 (br s, 1H, OH), 11.30

(s, 1H, NH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 115 (2xCH), 121 (CH), 121 (2 × CH), 127 (Cq), 130 (2 × CH), 135 (Cq), 139 (Cq), 140 (Cq), 146 (CH), 147 (Cq), 150 (2 × CH), 160 (Cq), 160 (CH), 160 (Cq), 165 (Cq); HRMS (EI-MS): calculated for $C_{19}H_{14}N_5O_2$ 344.11470 $[M+H]^+$ found 344.11440 $[M+H]^+$.

6.1.24. 1-[2-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-7-yl] piperidin-2-one **48**

Compound **48** was obtained following the general procedure **A** using piperidin-2-one which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/MeOH 98/2) as a yellowish solid in a 79% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 268–270 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3333, 1621, 1570, 1452, 1223, 1157, 807, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.89–1.95 (m, 4H, H_{PP}), 2.53 (t, 2H, J = 6.5 Hz, H_{PP}), 3.88 (t, 2H, J = 5.5 Hz, H_{PP}), 6.92 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.21 (d, 1H, J = 1.9 Hz, H_8), 8.40 (d, 2H, J = 8.7 Hz, H_{Ph}), 9.04 (d, 1H, J = 1.9 Hz, H_6), 9.57 (s, 1H, H_4), 10.08 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 20 (CH_2), 22 (CH_2), 32 (CH_2), 49 (CH_2), 115 (2xCH), 127 (CH), 127 (Cq), 130 (2 × CH), 135 (Cq), 144 (Cq), 146 (Cq), 150 (CH), 160 (Cq), 160 (Cq), 160 (CH), 170 (Cq); HRMS (EI-MS): calculated for $C_{18}H_{17}N_4O_2Na$ 343.11710 $[M+H]^+$ found 343.11700 $[M+H]^+$.

6.1.25. 1-[2-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-7-yl] piperazin-2-one **49**

Compound **49** was obtained following the general procedure **A** using piperazin-2-one which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) as a yellowish solid in a 78% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3495, 1639, 1566, 1448, 1261, 1161, 847, 708; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.10 (s, 2H, H_{Pr}), 3.16 (s, 1H, NH), 3.52 (s, 2H, H_{Pr}), 3.88 (s, 2H, H_{Pr}), 6.93 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.27 (d, 1H, J = 1.7 Hz, H_8), 8.40 (d, 2H, J = 8.6 Hz, H_{Ph}), 9.13 (d, 1H, J = 1.7 Hz, H_6), 9.58 (s, 1H, H_4), 10.11 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 42 (CH_2), 50 (CH_2), 50 (CH_2), 115 (2 × CH), 126 (CH), 127 (Cq), 130 (2 × CH), 135 (Cq), 143 (Cq), 146 (Cq), 150 (CH), 160 (Cq), 160 (CH), 160 (Cq), 168 (CO); HRMS (EI-MS): calculated for $C_{17}H_{16}N_5O_2$ 322.13040 $[M+H]^+$ found 322.13060 $[M+H]^+$.

6.1.26. 1-[2-(4-Hydroxyphenyl)pyrido[3,2-d]pyrimidin-7-yl] pyrrolidin-2-one **50**

Compound **50** was obtained following the general procedure **A** using pyrrolidin-2-one which afforded the attempted derivative after a purification under flash chromatography on silica gel (CH_2Cl_2/MeOH 99/1) as a pinkish solid in a 93% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3423, 1665, 1571, 1460, 1260, 1156, 806, 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11–2.19 (m, 2H, H_{Py}), 2.60 (t, 2H, J = 8.0 Hz, H_{Py}), 4.03 (t, 2H, J = 7.0 Hz, H_{Py}), 6.92 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.30 (s, 1H, H_8), 8.37 (d, 2H, J = 8.6 Hz, H_{Ph}), 9.50 (s, 2H, H_6 and H_4); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 17 (CH_2), 31 (CH_2), 47 (CH_2), 115 (2 × CH), 120 (CH), 127 (CH), 129 (2 × CH), 134 (Cq), 139 (Cq), 144 (Cq), 146 (Cq), 159 (CH), 160 (Cq), 160 (Cq), 174 (CO); HRMS (EI-MS): calculated for $C_{17}H_{15}N_4O_2$ 307.11895 $[M+H]^+$ found 307.11904 $[M+H]^+$.

6.1.27. 7-Chloro-2-(4-hydroxyphenylamino)pyrido[3,2-d] pyrimidine **51**

In a solution of dioxane containing (200 mg, 1.0 mmol) of compound **1**, (131.0 mg, 1.19 mmol, 1.2 eq.) of 4-hydroxyaniline was added and the reaction mixture was refluxed for 24 h. The solution was cooled, dioxane was removed under pressure and the crude material was purified by flash chromatography on silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) to afford **51** as an orange solid in a 60% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 232–234 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3474, 1607, 1445, 1198, 1072, 820, 714; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.73 (d, 2H, J = 8.5 Hz, H_{Ph}), 7.67 (d, 2H, J = 8.5 Hz, H_{Ph}), 8.13 (d, 1H, J = 1.5 Hz, H_8), 8.63 (d, 1H, J = 1.5 Hz, H_6), 9.17 (s, 1H, H_4), 9.26 (s, 1H, NH), 9.96 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 114 (2 × CH), 121 (CH), 131 (Cq), 131 (2 × CH), 134 (Cq), 134 (Cq), 145 (CH), 147 (Cq), 152 (Cq), 157 (Cq), 162 (CH); HRMS (EI-MS): calculated for $C_{13}H_{10}Cl^{35}N_4O$ 273.05430 $[M+H]^+$ found 273.05390 $[M+H]^+$.

6.1.28. (4-Methoxy-phenyl)-[7-(2-methoxy-phenyl)-pyrido[3,2-d] pyrimidin-2-yl]-amine **52**

Compound **52** was obtained from **51** as described for compound **25** using 2-hydroxyphenyl boronic acid. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) afforded the attempted product as an orange solid in 66% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp 262–264 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3433, 1602, 1434, 1213, 1105, 800, 725; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.73 (d, 2H, J = 8.6 Hz, H_{Ph}), 6.94–7.05 (m, 2H, H_{Ph}), 7.26–7.32 (m, 1H, H_{Ph}), 7.48 (d, 1H, J = 7.0 Hz, H_{Ph}), 7.71 (d, 2H, J = 8.6 Hz, H_{Ph}), 8.05 (s, 1H, H_8), 8.87 (s, 1H, H_6), 9.11 (s, 1H, H_4), 9.24 (s, 1H, NH), 9.77 (s, 1H, OH), 10.00 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 114 (2 × CH), 116 (CH), 119 (CH), 120 (CH), 123 (Cq), 130 (CH), 130 (CH), 131 (2xCH), 131 (Cq), 134 (Cq), 139 (Cq), 147 (Cq), 148 (CH), 152 (Cq), 154 (Cq), 157 (Cq), 162 (CH); HRMS (EI-MS): calculated for $C_{19}H_{15}N_4O_2$ 331.11950 $[M+H]^+$ found 331.12110 $[M+H]^+$.

6.1.29. (4-Hydroxy-phenyl)-[7-(3-hydroxy-phenyl)-pyrido[3,2-d] pyrimidin-2-yl]-amine **53**

Compound **53** was obtained from **51** as described for compound **25** using 3-hydroxyphenyl boronic acid. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) afforded the attempted product as an orange solid in 60% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3476, 1595, 1399, 1219, 1170, 887, 794; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.73 (d, 2H, J = 8.7 Hz, H_{Ph}), 6.87–6.91 (m, 1H, H_{Ph}), 7.23 (s, 1H, H_{Ph}), 7.30–7.35 (m, 2H, H_{Ph}), 7.72 (d, 2H, J = 8.7 Hz, H_{Ph}), 8.08 (d, 1H, J = 1.4 Hz, H_8), 8.93 (d, 1H, J = 1.4 Hz, H_6), 9.11 (s, 1H, H_4), 9.25 (s, 1H, NH), 9.70 (s, 1H, OH), 9.82 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 114 (CH), 114 (2 × CH), 116 (CH), 118 (CH), 120 (CH), 129 (CH), 130 (2 × CH), 131 (Cq), 135 (Cq), 137 (Cq), 140 (Cq), 145 (CH), 147 (Cq), 152 (Cq), 157 (Cq), 157 (Cq), 162 (CH); HRMS (EI-MS): calculated for $C_{19}H_{15}N_4O_2$ 331.11950 $[M+H]^+$ found 331.12000 $[M+H]^+$.

6.1.30. (4-Hydroxy-phenyl)-[7-(4-hydroxy-phenyl)-pyrido[3,2-d] pyrimidin-2-yl]-amine **54**

Compound **54** was obtained from **51** as described for compound **25** using 4-hydroxyphenyl boronic acid. Flash chromatography using silica gel (CH_2Cl_2/NH_3 99/1 then CH_2Cl_2/MeOH 98/2) afforded the attempted product as an orange solid in 80% yield. R_f: 0.2 (petroleum ether/EtOAc 5/95); mp > 268 °C; IR (ATR Diamond, KBr, cm^{-1}) ν 3453, 1599, 1359, 1210, 1171, 819, 719; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.74 (d, 2H, J = 8.6 Hz, H_{Ph}), 6.91 (d, 2H, J = 8.3 Hz, H_{Ph}) 7.69–7.80 (m, 4H, H_{Ph}), 8.06 (s, 1H, H_8), 8.97 (s, 1H, H_6), 9.11 (s, 1H, H_4), 9.21 (s, 1H, NH), 9.76 (s, 1H, OH), 9.87 (s, 1H, OH); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 114 (2 × CH), 116 (2 × CH), 120 (2 × CH), 126 (Cq), 127 (CH), 128 (2xCH), 131 (Cq), 134 (Cq), 139 (Cq), 145 (CH), 147 (Cq), 152 (Cq), 157 (Cq), 158 (Cq), 161 (CH); HRMS (EI-MS): calculated for $C_{19}H_{15}N_4O_2$ 331.11950 $[M+H]^+$ found 331.11900 $[M+H]^+$.

6.2. Kinase preparations and assays

Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 μg heparin/ml. *Buffer C*: 60 mM β-glycerolphosphate, 15 mM *p*-nitrophenylphosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1.0 mM phenylphosphate. Kinase activities were assayed in Buffer A or C, at 30 °C, at a final ATP concentration of 15 μM [10]. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. *CDK5/p25* (human, recombinant) was prepared as previously described. Its kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in the presence of 15 μM [γ-33P] 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 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid/liter of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid. *GSK-3αβ* (porcine brain, native) was assayed, as described for CDK5/p25 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine). GS-1 was synthesized by Millegen (Labege, France). *DYRK1A* (rat, recombinant, expressed in *Escherichia coli* as a GST fusion protein) was purified by affinity chromatography on glutathione–agarose and assayed as described for CDK5/p25 using myelin basic protein (1 mg/mL) as a substrate [11].

6.3. Molecular modelling

Hardware and software: molecular modelling studies were performed with the Schrodinger Molecular Modelling Suite 2012 update 2 [12] where Maestro is the interface piloting the diverse modules. Glide was used to dock ligands. Analysis and visualization tasks were performed within MOE software [13]. Calculations were run on a Linux station: Intel_ Xeon CPU W3670 @ 3.20 GHz.

Structure preparation:

Crystal structures were retrieved from the protein data bank:

1. DYRK1A with PDB code 2vx3 [14]. Subunit A was conserved.
2. CDK5 with PDB code 3O0G [15]. The most complete subunit A was conserved. Asparagine 144 was mutated to Aspartate in DFG motif to respect human sequence.
3. GSK3β with PDB code 1q41 [16]. The most complete subunit B was conserved again.

Structures were next prepared using the Protein Preparation Wizard workflow of the Schrodinger Molecular Modelling Suite. Proteins were preprocessed (hydrogen atoms added, incomplete residues filled), bond orders and connections of ligands were manually corrected. An exhaustive sampling was conducted regarding hydrogen bond assignment and the complex was finally refined by a minimization stage with a constraint to converge to a structure with an RMSD of 0.3 Å (OPLS2005 force field), essentially in order to remove steric clashes. Ligands, other than the one co-crystallized, were built within Accelrys Draw [17] and were submitted to Corina [18], a 3D structure generator. Next 3D structures were submitted to the LigPrep module of the Schrodinger Molecular Modelling Suite in order to take into account tautomerization and ionization via the Epik module. The resulting structures became the starting point for docking simulations.

Docking parameters: docking calculations were performed with extra precision. Ligand flexibility was taken into account and the option of sampling of ring conformation was activated.

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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.2014.04.055.

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