Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of novel 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines and their *N*-aryl analogues and evaluation of their inhibitory activity against Ser/Thr kinases

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ARTICLE INFO

Article history:
Received 2 September 2013
Revised 7 October 2013
Accepted 8 October 2013
Available online xxxx

Keywords:
Microwave-assisted chemistry
Dimroth rearrangement
Formamide degradation
Suzuki–Miyaura cross-coupling
Serine/threonine kinases inhibitors

ABSTRACT

The efficient synthesis of 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines and their *N*-aryl analogues is described. 3,5-Dibromopyridine was converted into 3-amino-6-bromofuro[3,2-*b*]pyridine-2-carbonitrile intermediate which was formylated with DMFDMA. Functionalization at position 7 of the tricyclic scaffold was accomplished, before or after cyclisation step, by palladium-catalyzed Suzuki–Miyaura cross-coupling while the pyrimidin-4-amines and *N*-aryl counterparts were synthesized by microwave-assisted formamide degradation and Dimroth rearrangement, respectively. The final products were evaluated for their potent inhibition of a series of five Ser/Thr kinases (CDK5/p25, CK1δ/ε, CLK1, DYRK1A, GSK3α/β). Compound **35** showed the best inhibitory activity with an IC_{50} value of 49 nM and proved to be specific to CLK1 among the panel of tested kinases.

In the course of our work aiming to synthesize new polyaromatic heterocycles with a potent inhibitory activity on serine/threonine kinases,[1] we wished to examine the potential of compounds bearing a pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine core. Indeed, a recent study performed by our group demonstrated that *N*′-(2-cyanofuro[3,2-*b*]pyridin-3-yl)-*N*,*N*-dimethylformimidamide **1** may undergo cyclisation to yield pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine **2** or its *N*-phenyl analogues **3**–**11** (series A)[2] by microwave-assisted formamide degradation[3] or Dimroth rearrangement,[4] respectively, as presented in Scheme 1.

Moreover, our recent investigations on the synthesis of 8-arylpyrido[3′,2′:4,5]thieno[3,2-*d*]pyrimidin-4-amines confirmed that Suzuki–Miyaura cross-coupling could be achieved on a thieno derivative of **1** to functionalize position 8 with an aryl moiety.[5] In light of these elements, and with the purpose of assessing the kinase inhibitory potential of pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine skeleton, we focused on the synthesis of a library of 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines and their *N*-aryl analogues. This Letter describes the development of a reliable strategy where microwave-assisted *N*,*N*-dimethylformamide dimethylacetal (DMFDMA)-mediated formylation,[6] Suzuki–Miyaura cross-coupling, formamide degradation and Dimroth rearrangement were associated. This protocol allowed the preparation of novel 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines (series B) and their *N*-aryl analogues (series C) for which relevant biological properties were expected.

The main part of the chemistry described in this Letter was realized under microwaves in an eco-compatible chemistry approach.[7] The evaluation of kinase inhibition was performed with five serine/threonine kinases: a cyclin-dependent kinase (CDK5/p25), casein kinase 1 (CK1δ/ε), cdc2-like kinase 1 (CLK1), dual-specificity, tyrosine phosphorylation regulated kinase (DYRK1A), glycogen synthase kinase 3 (GSK3α/β). The latter were chosen for their strong implication in various regulation processes, especially Alzheimer's disease (AD).[8]

Owing to the reactivity of intermediate **1**, we considered the synthesis of 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines (series B) and their *N*-aryl analogues (series C) from a brominated precursor **12** according to the retrosynthetic pathway presented in Scheme 2. In order to complete the synthesis of potentially bioactive products of series B and C, we initially considered the synthesis of the common precursor **12** of both series B and C, starting from commercially available 3,5-dibromopyridine **13**.

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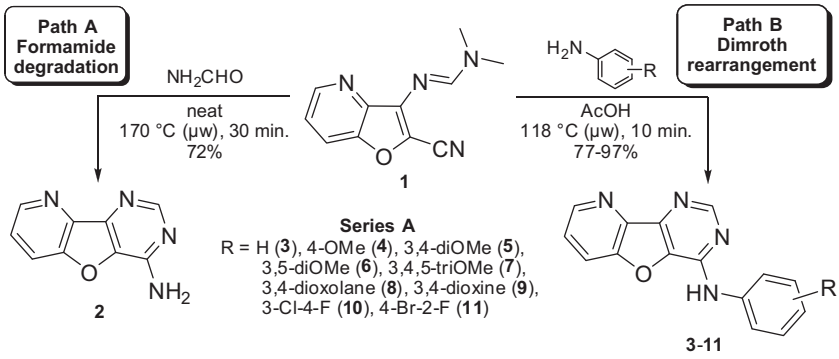
0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.10.019



Scheme 1. Previous work: synthesis of pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine **2** or its *N*-aryl analogues **3**–**11** (series A) by formamide degradation (path A) or Dimroth rearrangement (path B).

Some of the steps of this previously described synthetic sequence[9] were optimized using microwave technology as presented in Scheme 3.

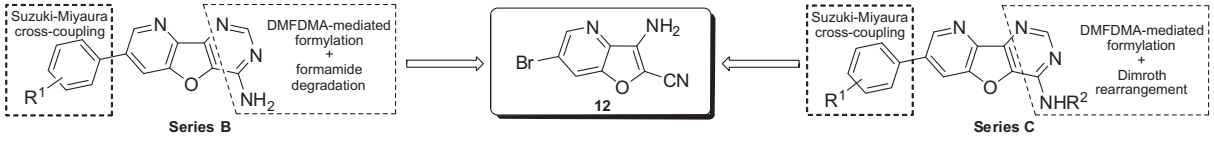
3,5-Dibromopyridine **13** was first desymmetrized into 3-bromo-5-methoxypyridine **14** in an excellent 88% yield using an excess of sodium methoxide in warm DMF. Optimization of this reaction by microwave irradiation at atmospheric pressure reduced the reaction time from 24 hours for conventional heating to 2 h in microwave oven. In the presence of boron tribromide in DCM at low temperature, 3-bromo-5-methoxypyridine **14** was efficiently deprotected into 5-bromopyridin-3-ol **15** in good 81% yield. Regioselective iodination of compound **15** was achieved with iodine in the presence of sodium carbonate, in water at room temperature, to afford the iodo intermediate **16** in good yield. The latter was successfully converted into corresponding 3-pyridyloxyacetonitrile derivative **17** using bromoacetonitrile. Substitution of iodine by a cyano group using copper(I) cyanide afforded the intermediate **18**. Finally, a microwave-assisted cyclisation of compound **18** in the presence of potassium carbonate in warm DMF furnished 3-amino-6-bromofuro[3,2-*b*]pyridine-2-carbonitrile **12** in 31% overall yield.

We then performed the synthesis of 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines (series B). The absence of structure–activity relationship features among the studied pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines series prompted us to employ the Topliss scheme for selecting substitution patterns on the aromatic substituent.[10] In order to accomplish the synthesis with the best overall yields, a previous study based on the synthesis of thieno analogues[5] led us to propose the following functionalization sequence: DMFDMA-mediated formylation, palladium-catalyzed Suzuki–Miyaura cross-coupling, and cyclisation with formamide. Accordingly, precursor **12** was reacted with DMFDMA using microwave irradiation at atmospheric pressure, and converted into 6-bromodimethylformimidamide derivative **19** in 87% yield along with traces of the 6-bromoformimidate **20**. Functionalization at position 7 of the intermediate **19** into the aryl adducts **21**–**28** was completed by a microwave-assisted Suzuki–Miyaura cross-coupling in the presence of a catalytic amount of [1,1′-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) dichloromethane complex (for cyanoamidines **21**–**27**) or tetrakis(triphenylphosphine)palladium(0) (for cyanoamidine **28**), the appropriate phenylboronic acid, and sodium carbonate at 150 °C as shown in Scheme 4. Noteworthy, the coupling reaction with some of the haloarylboronic acids gave moderate yields due to purification problems while alkyl- and alkoxyphenylboronic acids gave coupling products in good to excellent yields. Cyclisation of cyanoamidine intermediates **21**–**28** into the final 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **29**–**36** was conducted according to our previously described methodology using a high temperature microwave-assisted degradation of formamide in moderate yields (Table 1).[3]

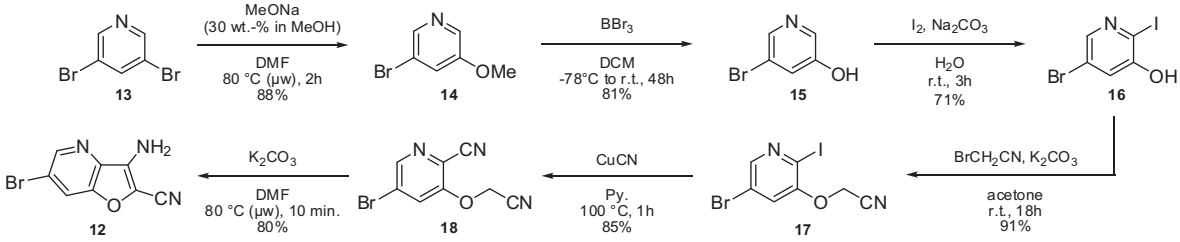
Based on preceding work of several groups demonstrating the importance of associating the 3,4-benzodioxolane ring to kinase inhibitors,[11] we decided to conceive series C from the structures of products **28** and **36**. Therefore the foreseen molecules (**39**–**47** in Table 2) were designed with a constant 3,4-benzodioxolane moiety at position 7 while modulations were achieved at position 4 of the pyrimidin-4-amine ring. In an attempt to optimize the overall yields, we investigated the best sequence for formylation, Suzuki–Miyaura cross-coupling and Dimroth rearrangement. Activated anilines bearing a methoxy substituent are known for improving Dimroth rearrangement,[4] we therefore chose to use 3,4-dimethoxyaniline for this short study. As presented in Scheme 5, path B (DMFDMA-mediated formylation + Suzuki–Miyaura cross-coupling + Dimroth rearrangement) gave the best overall yield for the conversion of precursor **12** into product **42**.

Conforming to this optimized sequence, intermediate **28** was reacted with various anilines in refluxing acetic acid to provide products **39**–**47** in moderate to excellent yields (Table 2) as presented in Scheme 6.

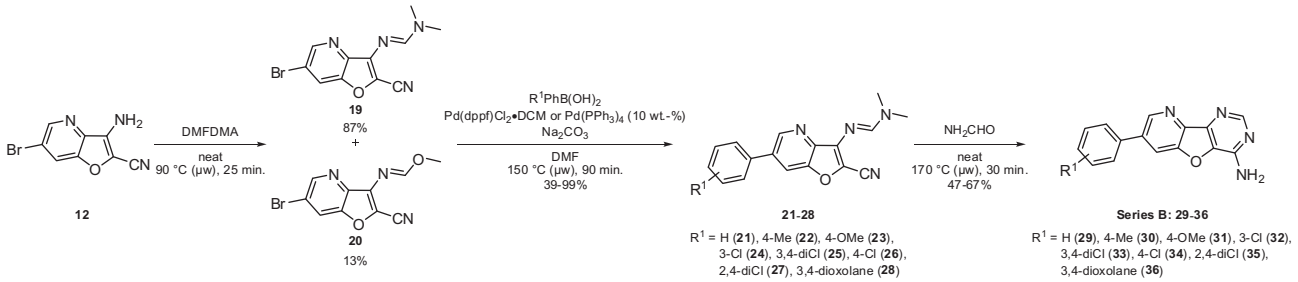
Products of series A (**2**–**11**), series B (**29**–**36**) and series C (**39**–**47**) were tested on five different in vitro kinase assays (CDK5/p25, CK1δ/ε, GSK3α/β, DYRK1A and CLK1) to evaluate their inhibition potency.[12–14] All compounds were first tested at a final concentration of 10 μM. 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



Scheme 2. Retrosynthetic pathway and access to novel 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines and *N*-arylpyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines from precursor **12**.

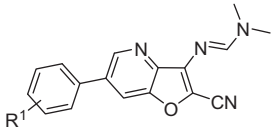
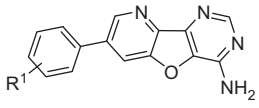


Scheme 3. Derivatization sequence of 3,5-dibromopyridine **13** into 3-amino-6-bromofuro[3,2-*b*]pyridine-2-carbonitrile **12**.



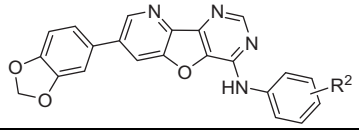
Scheme 4. Microwave-assisted synthesis of cyanoamidine **19** with DMFDMA, aryl adducts **21**–**28** by palladium-catalyzed Suzuki–Miyaura cross-coupling, and 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **29**–**36** by formamide degradation. For detailed yields, see Table 1.

Table 1
Yields for the synthesis of the aryl adduct intermediates **21**–**28** and 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **29**–**36** (series B)

Product 	R^1	Yield[a] (%)	Product 	R^1	Yield[a] (%)
21	H	99	**29**	H	51
22	4-Me	79	**30**	4-Me	47
23	4-OMe	89	**31**	4-OMe	57
24	3-Cl	76	**32**	3-Cl	53
25	3,4-DiCl	48	**33**	3,4-DiCl	61
26	4-Cl	39	**34**	4-Cl	56
27	2,4-DiCl	44	**35**	2,4-DiCl	54
28	3,4-Dioxolane	66	**36**	3,4-Dioxolane	67

[a] Isolated yield.

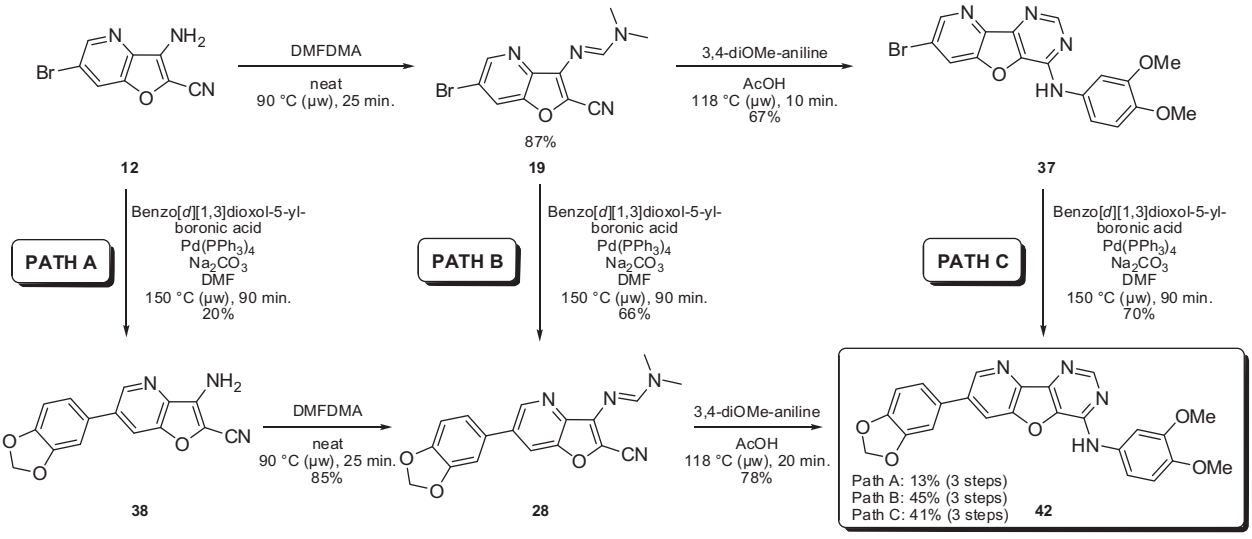
Table 2
Isolated yields for 7-substituted *N*-arylpyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **39**–**47**

Product 	R^2	Reaction time (min)	Yield[a] (%)
39	H	20	91
40	4-Me	45	99
41	4-OMe	20	89
42	3,4-DiOMe	20	78
43	3,4,5-TriOMe	40	67
44	3,4-DiCl	120	36
45	4-Cl	40	53
46	3-Cl-4-F	120	75
47	3,4-Dioxolane	80	88

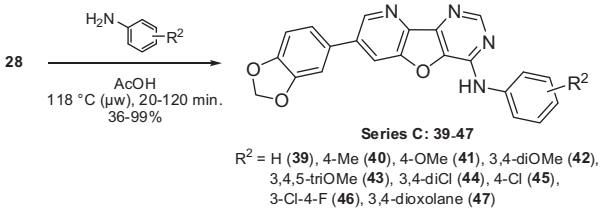
[a] Isolated yield.

IC_{50} values were determined from the dose-response curves (Sigma-Plot).

Results given in Table 3 demonstrated that none of the tricyclic derivatives of series A and C showed affinity against the series of



Scheme 5. Optimization of the order of reaction sequence for DMFDMA-mediated formylation, palladium-catalyzed Suzuki–Miyaura cross-coupling, and Dimroth rearrangement.



Scheme 6. Microwave-assisted synthesis of 7-substituted *N*-arylpyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **39**–**47** by Dimroth rearrangement. For detailed yields and reaction times, see Table 2.

five kinases. The most promising results were obtained with products from the series B (**29**–**36**). Most of them displayed a rather good inhibition of both CLK1 and DYRK1A kinases with micromolar or submicromolar IC_{50} values. On a general aspect, series B derivatives (**29**–**36**) were completely inactive toward CDK5/p25, CK1δ/ε and GSK3α/β.

Among the molecules of series B, three pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines (**29**, **30**, and **36**) were judged relatively active with submicromolar IC_{50} against CLK1 and with values against kinase DYRK1A in the micromolar range. The IC_{50} values of these three dual-inhibitors were judged similar with a slightly better activity for **36** (IC_{50} values of 0.32 and 2.3 μM for CLK1 and DYRK1A, respectively).

Among the tested compounds, the pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine substituted by a 2,4-dichlorophenyl group (**35**) can be considered as the most active product because it was the only one able to inhibit two kinases in the submicromolar range (IC_{50} values of 0.049 and 0.60 μM for CLK1 and DYRK1A, respectively).

With regard to this set of results on series A, B and C, it appears that substitution by an *N*-aryl moiety in position 4 of the pyrimidine ring reduces affinities for kinases. In contrast, the free amine and substitution at position 7 might play an important role in the interaction of the molecules with the target. In our case, the use of Topliss list efficiently overcame the absence SAR studies. Hence, it demonstrated its utility in the rapid discovery of a new hit compound (e.g., compound **35**).

CLK1 is one of the four isoforms of the cdc2-like kinase family. It was described that inhibitors of CLK1 could prove to be useful agents in disease phenotypes characterized by abnormal splicing. In this sense CLK inhibitors may alter the splicing of microtubule-associated protein tau implicated in Alzheimer's disease and Parkinson's disease.[15]

Table 3
Kinase inhibitory activity of pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine **2** and its *N*-aryl analogues **3**–**11** (series A), 7-substituted pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **29**-**36** (series B) and 7-substituted *N*-arylpyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amines **39**–**47** (series C)

Series	Products	Ser/Thr kinase[a,b]				
		CDK5/p25	CK1δ/ε	CLK1	DYRK1A	GSK3α/β
A	**2**–**11**	>10	>10	>10	>10	>10
B	**29**	>10	>10	0.69	3.1	>10
	30	>10	>10	0.61	4.4	>10
	31	>10	>10	>10	>10	>10
	32	>10	>10	1	>10	>10
	33	>10	>10	>10	>10	>10
	34	—[c]	—[c]	—[c]	—[c]	—[c]
	35	>10	>10	0.049	0.6	>10
	36	>10	>10	0.32	2.3	>10
C	**39**–**47**	>10	>10	>10	>10	>10

[a] IC_{50} values are reported in μM. The most significant results are presented in bold.
[b] Kinases activities were assayed in triplicate. Typically, the standard deviation of single data points was below 10%.
[c] Not determined.

In view of the results of this preliminary study, we consider that the tricyclic pyrido[2′,3′:4,5]furo[3,2-*d*]pyrimidin-4-amine analogues (series B) constitute a promising source of inspiration for the synthesis of novel bioactive molecules. Synthetic transformations will be explored and factors governing their dual activity toward CLK1 and DYRK1A will be further investigated.

Acknowledgments

This work was supported by a Ph.D. grant from the Région Haute-Normandie (Y.L.). We thank the LABEX SynOrg (ANR-11-LABX-0029) and AI-Chem Channel program for financial support. We acknowledge Milestone s.r.l. (Italy) for technical support and Anton Paar GmbH (Graz, Austria) for provision of the single-mode microwave reactor (Monowave 300). It was also supported by the EEC FP7-KBBE-2012 BlueGenics grant (L.M.), 'Institut National contre le Cancer' (INCa) GLIOMER program and the 'Fonds Unique Interministériel' (FUI) PHARMASEA project (L.M.).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.10.019.

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