2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Synthesis of bioactive 2-(arylamino)thiazolo[5,4-f] quinazolin-9-ones via Hügershoff reaction or Cu catalyzed intramolecular C-S bond formation

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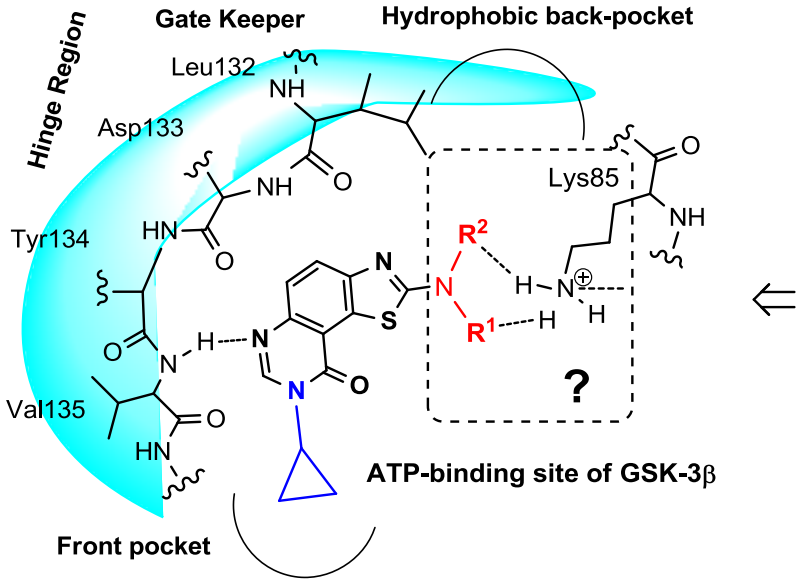
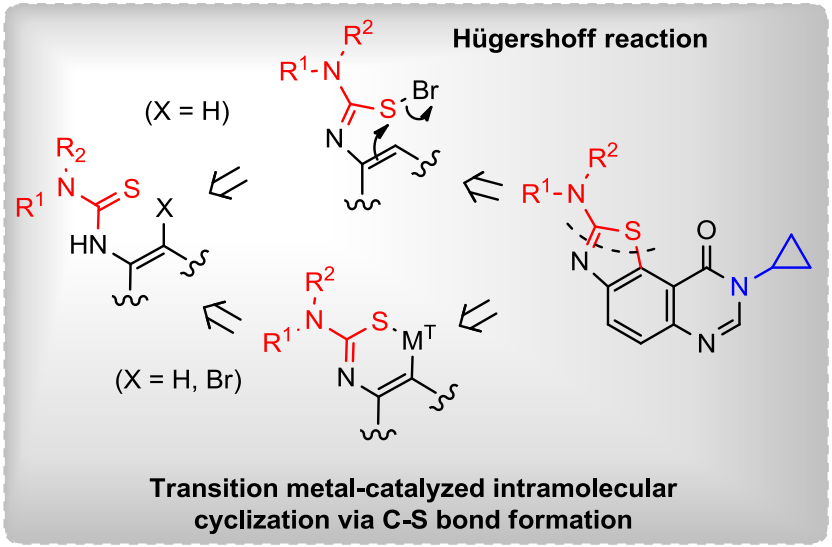




Abstract: A library of thirty eight novel thiazolo[5,4-*f*]quinazolin-9(8*H*)-one derivatives (series **8**, **10**, **14** and **17**) was prepared via the Hügershoff reaction and a Cu catalyzed intramolecular C-S bond formation, helped by microwave-assisted technology when required. The efficient multistep synthesis of the key 6-amino-3-cyclopropylquinazolin-4(3*H*)-one (**3**) has been reinvestigated and performed on a multi-gram scale from the starting 5-nitroanthranilic acid. The inhibitory potency of the final products was evaluated against five kinases involved in Alzheimer's disease and showed that some molecules of the **17** series described in this paper are particularly promising for the development of novel multi-target inhibitors of kinases.

Keywords: Hügershoff reaction; thiazolo[5,4-f]quinazolin-9(8H)-ones; microwave-assisted synthesis; protein kinases

Introduction

Our research group is mainly invested in the synthesis of C,N,S-containing bioactive molecules able to modulate the activity of deregulated kinases (CDK5, GSK-3, CLK1, CK1 and the dual-specificity kinase DYRK1A) involved to some extent in Alzheimer's disease (AD). Among them some thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones (Figure 1) have been revealed of particular interest in the design of multi-target-directed ligands (MTDLs), a new strategy for the development of new tools against neurodegenerative diseases.

The target molecules were thiazolo[5,4-*f*]quinazolin-9-ones substituted in position N-8 by a cyclopropyl chain (Figure 1).

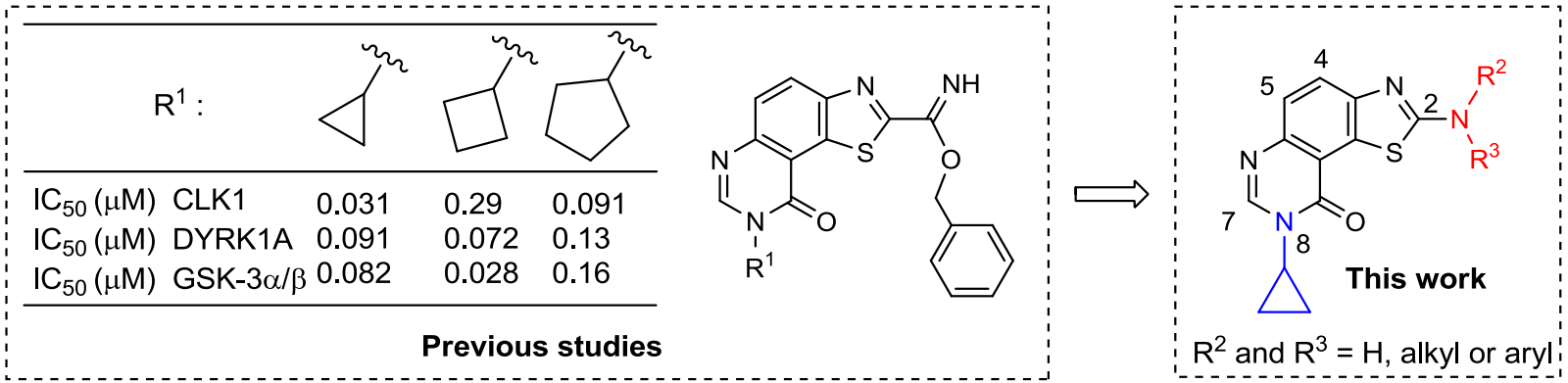
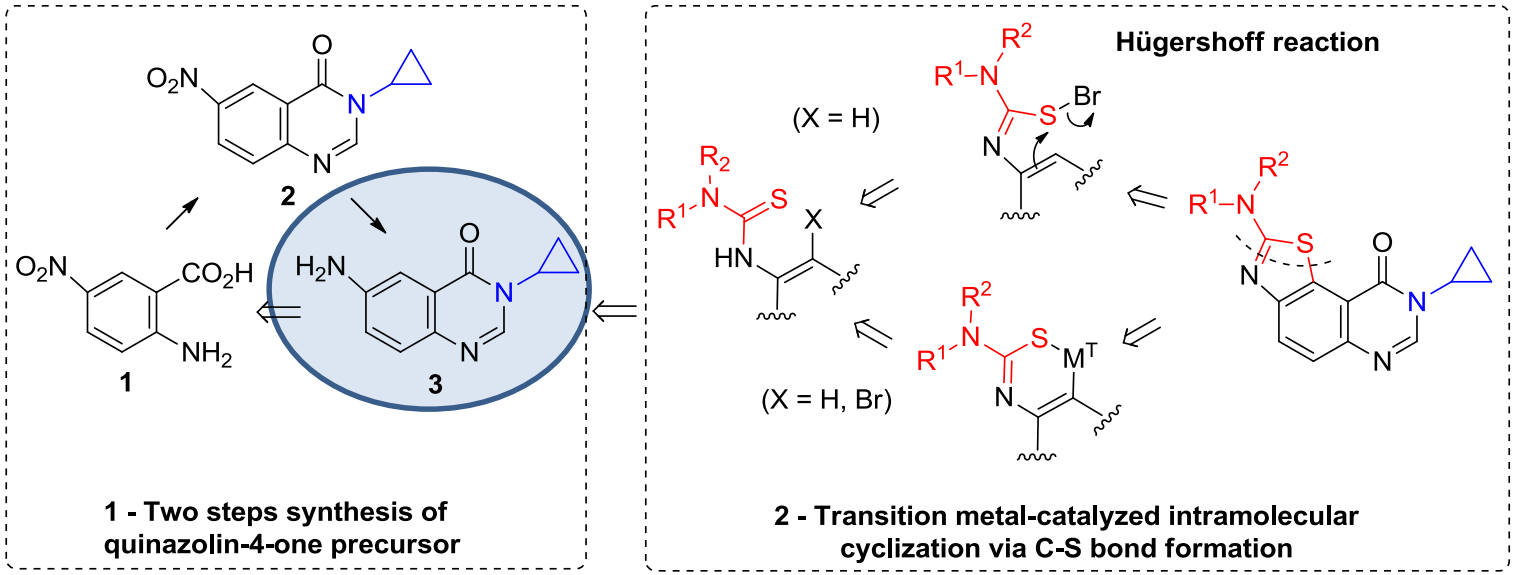


Figure 1. General formula of lead kinases inhibitors previously described [1-5] and new molecules targeted.

Results and discussion : *Envisioned retrosynthetic pathway and the key 6-amino-3-cyclopropylquinazolin-4(3H)-one* (**3**).

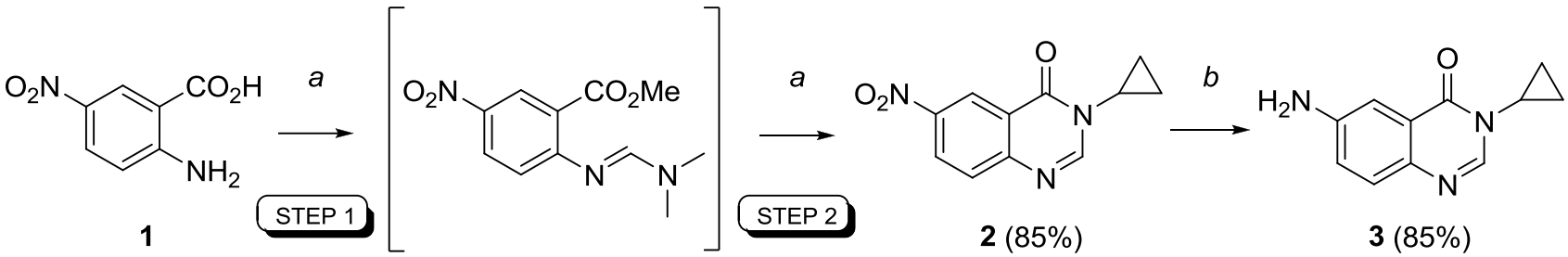
The first planned strategy uses the Hügershoff reaction, a bromine-mediated cyclization process involving electrophilic addition. The second route imagined concerns a metal catalyzed intramolecular C-S bond formation on the synthesis of variously 2-substituted benzothiazoles from thiobenzanilides (Scheme 1).



Scheme 1. Envisioned retrosynthetic pathway for the synthesis of the target products, *via* Hügershoff reaction or transition metal-catalyzed intramolecular C-S bond formation.

Results and discussion: *Synthesis of the key 6-amino-3-cyclopropylquinazolin-4(3H)-one* (**3**).

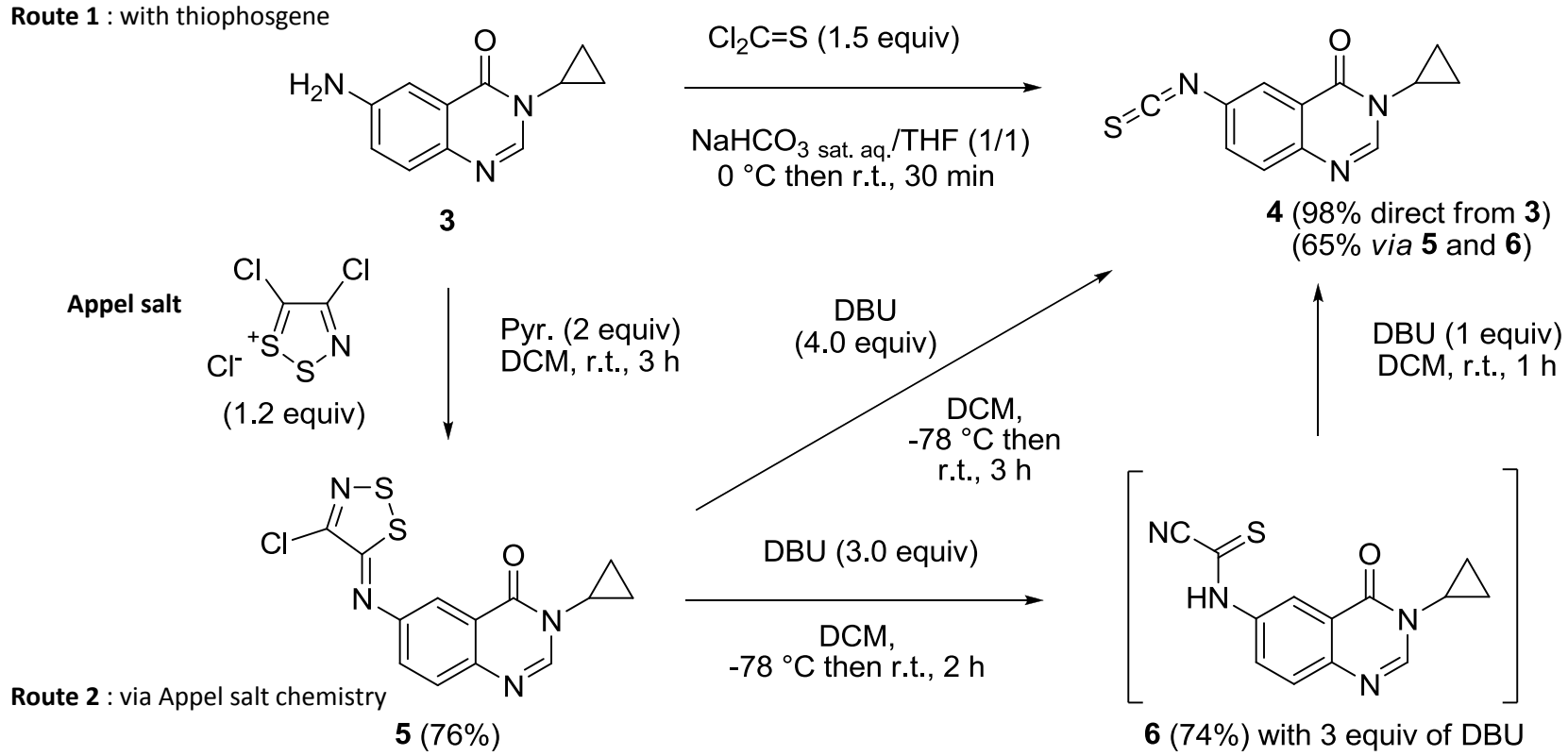
The key 6-amino-3-cyclopropylquinazolin-4(3*H*)-one (**3**) was obtained via an efficient microwave-assisted MCR procedure.



Scheme 2. Sequential MCR procedure for convenient synthesis of 6-amino-3-cyclopropylquinazolin-4(3*H*)-one (**3**) from **1**. *Reagents and conditions:* (*a*) Step 1: DMFDMA (2.5 equiv), DMF, 100 °C (µw), 15 min; Step 2: Cyclopropylamine (1.1 eq), AcOH, 100 °C (µw), 15 min; 85%; (*b*) HCO_2NH_4 (5.0 equiv), Pd/C (10%), EtOH, 85 °C (µw), 15 min; 85%.

Results and discussion : *Synthesis of 2-aminoarylthiazolo[5,4-f]quinazolin-9-ones via Hügershoff reaction*

Synthetic routes for access to the key isothiocyanate **4.**



Results and discussion : *Synthesis of thiourea **7a-l** from amine **3** or from isothiocyanate **4**.*

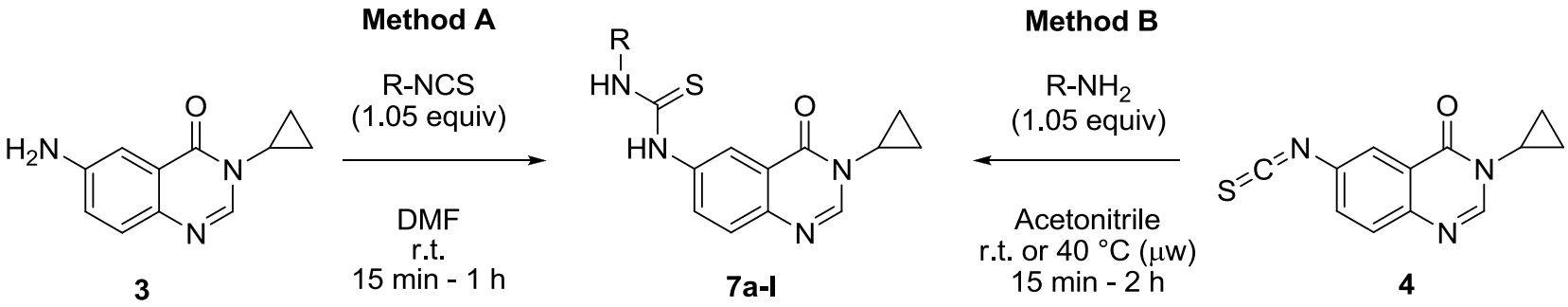
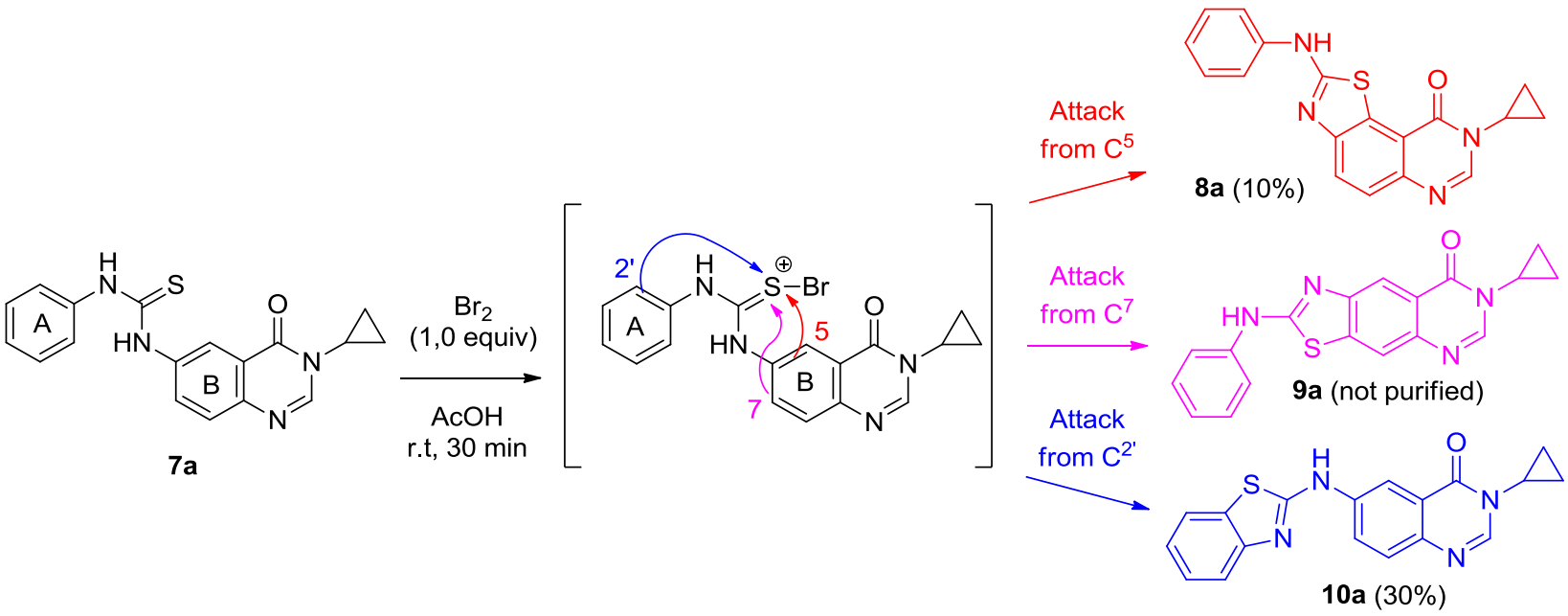


Table 1. Chemical structures and yields obtained for the synthesis of series **7a–l**.

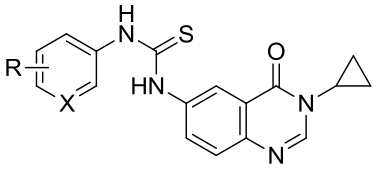
-R [a]	Compound	Method	Time (h)	Yield [b] (%)
Ph	**7a**	A/B	1/2	84/80
4-Cl-C_6H_4	**7b**	A	1	91
4-F-C_6H_4	**7c**	B	2	90
4-CF_3-C_6H_4	**7d**	B[c]	1	84
4-NO_2-C_6H_4	**7e**	A	0.5	74
2,4-diCl-C_6H_3	**7f**	B	1	89
3-Py	**7g**	B[c]	2	89
4-Me-C_6H_4	**7h**	A	2	89
4-OMe-C_6H_4	**7i**	A	2	87
4-NMe_2-C_6H_4	**7j**	B	10 min	87

Results and discussion : *Synthesis of 2-aminoarylthiazolo[5,4-f]quinazolin-9-ones via Hügershoff reaction.*



The cyclization was not regioselective. The phenyl group (A) of the starting diaryl thiourea **7a** possessed the most electron-rich *ortho*-carbon, compared to the quinazolin-4-one ring B. Compounds **10** are the major products.

Results and discussion : *Synthesis of 2-aminoarylthiazolo[5,4-f]quinazolin-9-ones via Hügershoff reaction.*

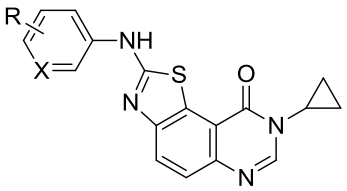


7b-g

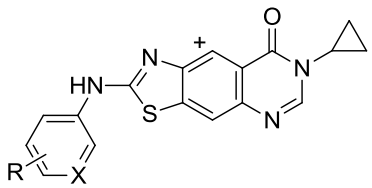
R = 4-Cl, 4-F, 4-CF_3, 4-NO_2, 2,4-diCl
X = CH or N (R = H)

AcOH
r.t., 1-4 h

Br_2
(1.0 equiv)

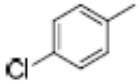
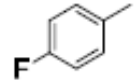
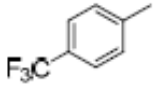
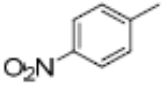
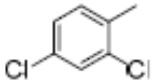


8b-g (45-90%)



9b-e (traces, not purified)

Table 2. Chemical structures and yields obtained for the synthesis of series **8a–g** via the Hügershoff reaction.

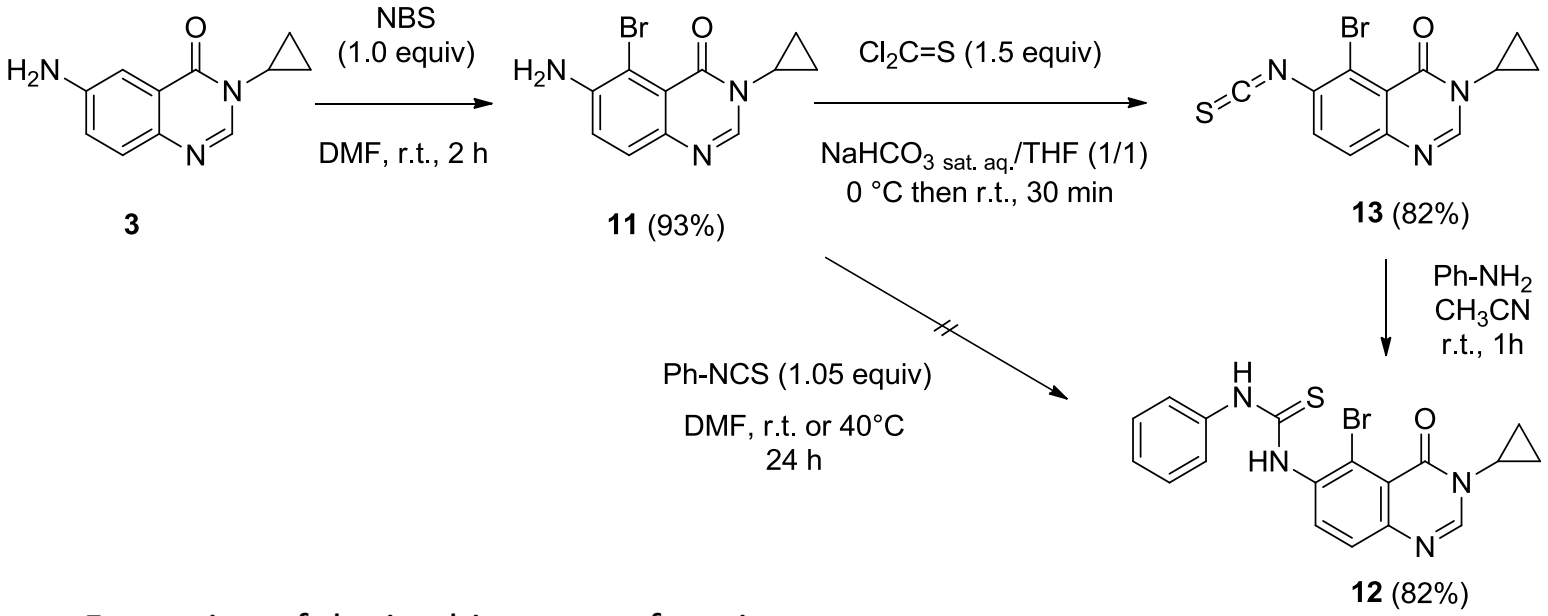
R, X	Compound	Time (h)	Yield [a] (%)
	**8a**	0.5	10 [b,c]
	**8b**	1	- [d]
	**8c**	1	45 [b]
	**8d**	1	79 [b]
	**8e**	4	86 [b]
	**8f**	1.5	90
	**8g**	4	80

[a] Isolated yield. [b] Traces of compounds of the 9 series were detected but not purified

Electron-deficient phenyl substituents are present in A cycle of the thiourea derivatives.

Results and discussion : *Synthesis of 2-aminoaryl- and 2-aminoalkylthiazolo[5,4-f]quinazolin-9-ones via Metal Catalyzed C-S Bond Formation.*

Synthesis of thiourea **12** via condensation of aniline with isothiocyanate **13**.

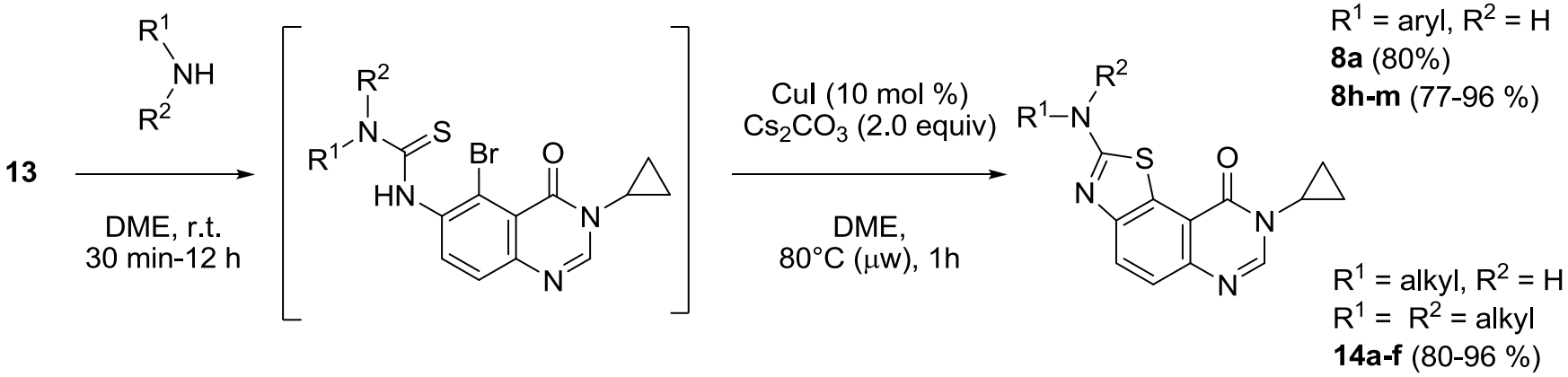


Formation of the isothiocyanate function was performed before bromination of the skeleton

Results and discussion : *Synthesis of 2-aminoaryl- and 2-aminoalkylthiazolo[5,4-f]quinazolin-9-ones via Metal Catalyzed C-S Bond Formation*

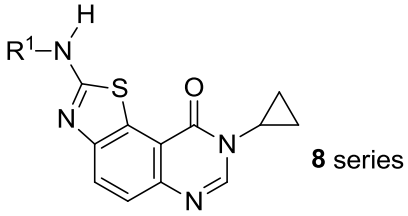
A sequential one-pot version of the transformation of isothiocyanate **13** into the tricyclic arene **8** series was considered.

This approach required the complete conversion of the starting material **13** into the corresponding thiourea intermediate (in square brackets) before addition of other reagents (CuI and Cs_2CO_3) and microwave-assisted cyclization by heating for 1 h.



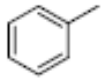
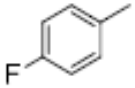
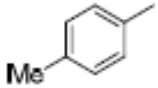
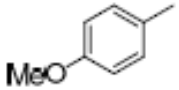
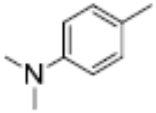
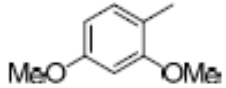
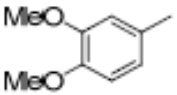
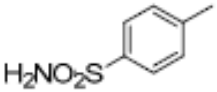
Results and discussion : *Synthesis of 2-aminoaryl- and 2-aminoalkylthiazolo[5,4-f]quinazolin-9-ones via Metal Catalyzed C-S Bond Formation.*

Depending on the nature of aniline, the conversion time can vary from 30 min (electron-rich compounds *e.g.* 4-OMe, 3,4-diOMe or 4-NMe_2 anilines) to an overnight stirring (12 h).



8 series

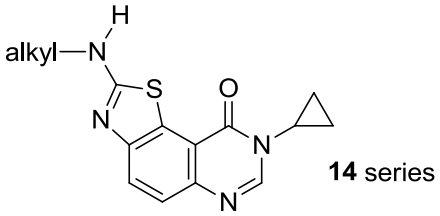
Because electron-poor anilines required several hours for completion of the first part of the reaction, the Hügershoff reaction was found to be more efficient in these cases (products **8d–f**).

-R^1	-R^2	Compound	Time [a] (h)	Yield [b] (%)
	H	**8a**	1	80
	H	**8c**	2	96
	H	**8h**	1	89
	H	**8i**	0.5	88
	H	**8j**	0.5	87
	H	**8k**	0.5	86
	H	**8l**	0.5	90
	H	**8m**	12	77

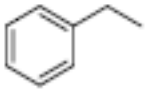
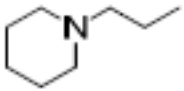
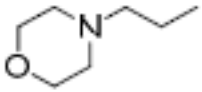
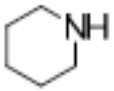
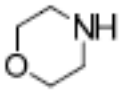
[a] Time of the conversion of 13 into the corresponding thioureas (step 1); [b] Isolated yield.

Results and discussion : *Synthesis of 2-aminoaryl- and 2-aminoalkylthiazolo[5,4-f]quinazolin-9-ones via Metal Catalyzed C-S Bond Formation.*

Microwave-assisted heating at 80 °C for 1 h complete the sequence to afford the expected cyclized compounds (series **14a–f**) in very good yields (80%–96%, see Table 5).

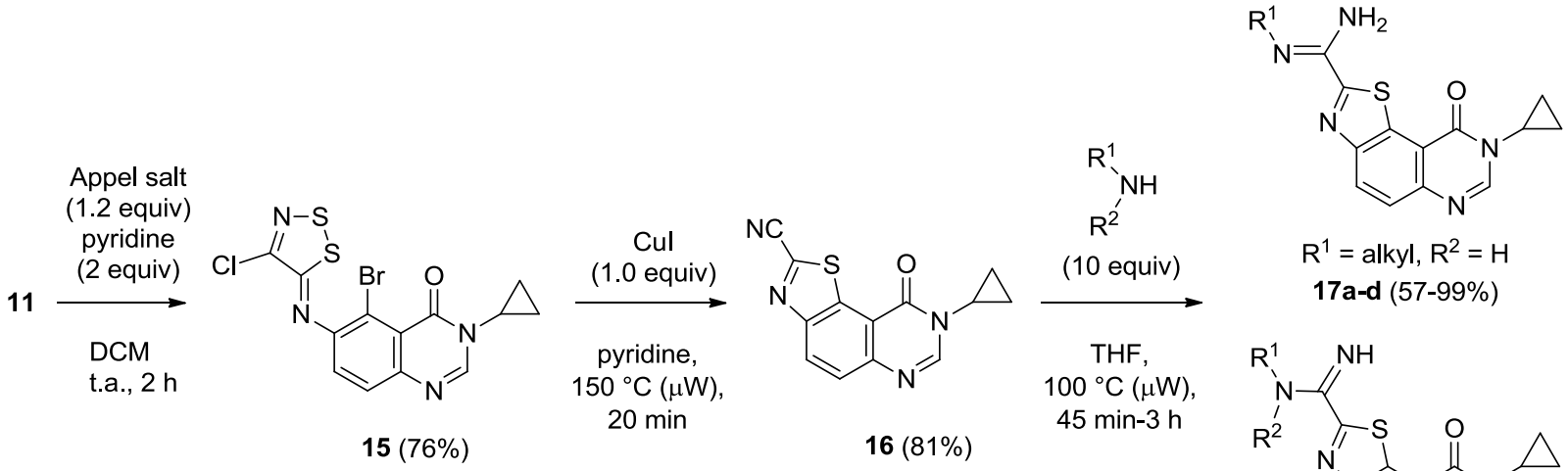


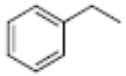
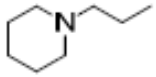
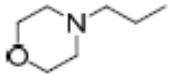
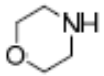
14 series

-R^1	-R^2	Compound	Time [a] (h)	Yield [b] (%)
	H	**14a**	0.5	80
	H	**14b**	0.5	96
	H	**14c**	0.5	89
	H	**14d**	0.5	88
		**14e**	0.5	87
		**14f**	0.5	86

[a] Time of the conversion of 13 into the corresponding thioureas (step 1); [b] Isolated yield.

Results and discussion : *Synthesis of carboximidamides **17a-f** from brominated amine **11**.*



-R^1	-R^2	Compound	Time [a] (min)	Yield [b] (%)
	H	**17a**	45	82
	H	**17b**	45	99
	H	**17c**	45	87
	H	**17d**	45	57
		**17e**	90	60
		**17f**	180	54

[a] Time of the conversion of 16 into the corresponding carboximidamides; [b] Isolated yield.

- Regio-selective intramolecular C-S coupling-reaction, catalyzed by CuI.

- A one-pot sequential process helped by microwave-assisted heating. It allowed the convenient synthesis of new thiazoloquinazolin-9-ones.

Results and discussion : Kinase inhibitory activity[a,b,c] of the thiazolo[5,4-*f*]quinazoline series (**7a-l**, **8a-m**, **10a** and **10l**, **14a-f** and **17a-f**).

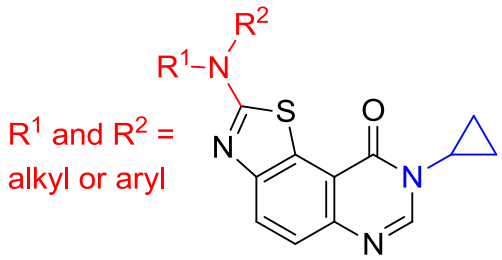
Compounds	CDK5/p25	CK1d/e	CLK1	DYRK1A	GSK-3a/b
7a-l	>10	>10	>10	>10	≥10
8a	>10	>10	3.4	>10	≥10
8c-f	>10	>10	>10	>10	≥10
8g	>10	>10	1.7	>10	≥10
8h-i	>10	>10	>10	>10	≥10
8j	>10	>10	1.3	2.0	7.3
8k-m	>10	>10	>10	>10	≥10
10a	>10	>10	5.2	4.8	≥10
10l	>10	>10	>10	>10	≥10
14a	>10	>10	3.4	>10	>10
14b	>10	>10	>10	>10	≥10
14c	>10	>10	>10	>10	≥10
14d	>10	>10	8.1	8.2	≥10
14e	>10	>10	5.3	>10	≥10
14f	>10	>10	>10	>10	≥10
17a	>10	>10	1.0	0.67	0.4
17b	>10	>10	0.29	4.4	1.1
17c	>10	>10	2.3	3.2	2.8
17d	>10	>10	2.1	2.9	3.9
17e	>10	1.9	0.38	0.14	0.23
17f	>10	>10	0.61	0.82	0.49
Harmine	>10	1.5	0.026	0.029	>10

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 to 10 µM), and IC_{50} values were determined from the dose-response curves (Sigma-Plot). *Harmine* is a β-carboline alkaloid known to be a potent inhibitor of DYRK1A. It was also tested as positive control and its IC_{50} values were compared to those obtained for the compounds under study.

The two most active molecules

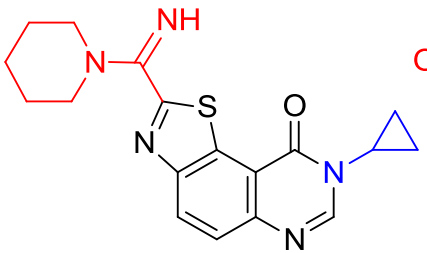
[a] IC_{50} values are reported in µM. The most significant results are presented in bold; [b] Kinases activities were assayed in triplicate. Typically, the standard deviation of single data points was below 10%.

Results and discussion : The two most active molecules : **17e** and **17f**



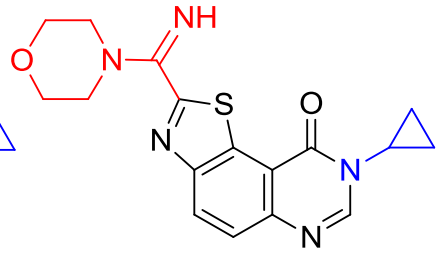
7, **8**,**10** and**14s**eries

Inactive compounds on
CDK5, CK1,CLK1, DYRK1A, GSK3



17e

CLK1 IC_{50} (μM) : 0.38
DYRK1A IC_{50} (μM) : 0.14
GSK3α/β IC_{50} (μM) : 0.23



17f

CLK1 IC_{50} (μM) : 0.61
DYRK1A IC_{50} (μM) : 0.82
GSK3α/β IC_{50} (μM) : 0.49

Comparing the results of 2-aminosubstituted derivatives and their carboximidamides analogs, it seems rather obvious that having such molecular scafolds with submicromolar affinities for various kinases is related to the presence **of carboximidamide or carboximidate functions** that result from the substitution of a carbonitrile group itself present in position 2 of the thiazole.

Conclusion

- A library of 38 novel 8-aminoaryl thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones (**8**, **10**, **14** and **17** series) has been prepared, using microwave-assisted technology:

- An efficient multistep synthesis of the key 6-amino-3-cyclopropylquinazolin-4(3*H*)-one (**3**) was developed and optimized to the multigram scale.
- The Hügershoff reaction was re-investigated under microwaves.
- A CuI catalyzed ligand-free intramolecular C-S bond formation was also developed.

- Molecules of the **8**, **10** and **14** series described in this paper are not pertinent for the development of kinases inhibitors.

- The most active compounds are carboximidamides analogues (**17** series) of the target compounds that shown submicromolar IC_{50} values for CLK1, DYRK1A and GSK-3α/β over the other tested enzymes.

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For more complete information see :

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Acknowledgments

Financial support from the MESR (French Ministère de l'Enseignement Supérieur & de la Recherche) is gratefully acknowledged for the doctoral fellowships to D.H.. We thank the LABEX SynOrg (ANR-11-LABX-0029) for financial support. We also acknowledge Milestone S.r.l. (Italy) for provision of multi-mode microwave reactor (Start S^{TM}) and for technical support. This research was partly supported by grants from the 'Fonds Unique Interministériel" (FUI) TRIAD (LM) projects, the "Fondation Jérôme Lejeune" (LM), and an FP7-KBBE-2012 grant (BlueGenics) to LM.