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Late-stage C-H Arylation of Thiazolo[5,4-*f*]quinazolin-9(8*H*)-one Backbone: Synthesis of an Array of Potential Kinase Inhibitors

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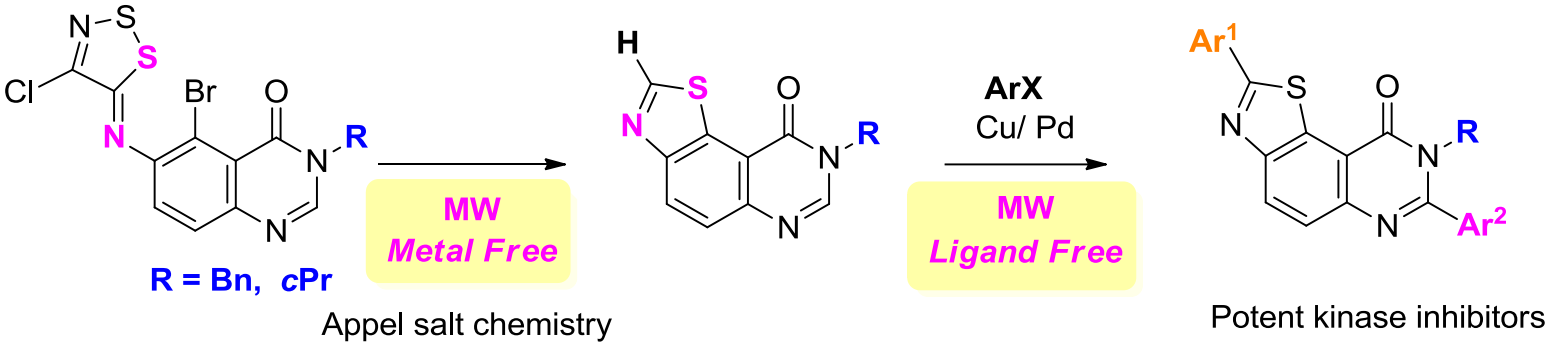
Graphical Abstract

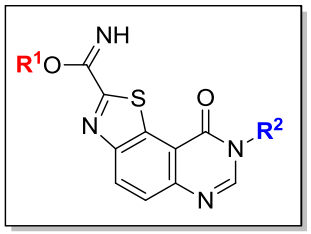


Abstract: Driven by the need of structural modification to establish structure–activity relationships, selective functionalization of thiazolo[5,4-*f*]quinazolin-9(8*H*)-one was developed through sequential activation of C-H bonds to furnish diarylated compounds . This strategy allows the regioselective C2 and C7 arylation by a judicious choice of coupling partners and bases, requiring no additional ligands or directing groups.
A more eco-friendly synthesis of thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones was also described giving access to these aforementioned compounds in a facile manner.

Keywords: thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones; microwave-assisted synthesis; C-H arylation; C-H activation; DYRK kinases inhibitors

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)[1]. 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 [2].



R^1 = Bn
R^2 = Cyclopropyl
IC_{50} CLK1 = 60 nM
IC_{50} DYRK1A = 60 nM
IC_{50} GSK3 = 100 nM

R^1 = Me
R^2 = Cyclopropyl
IC_{50} CLK1 = 31 nM
IC_{50} DYRK1A = 91 nM
IC_{50} GSK3 = 82 nM

R^1 = Me
R^2 = Benzyl
IC_{50} CLK1 = 500 nM
IC_{50} DYRK1A = 300 nM
IC_{50} GSK3 = 220 nM

Figure 1.

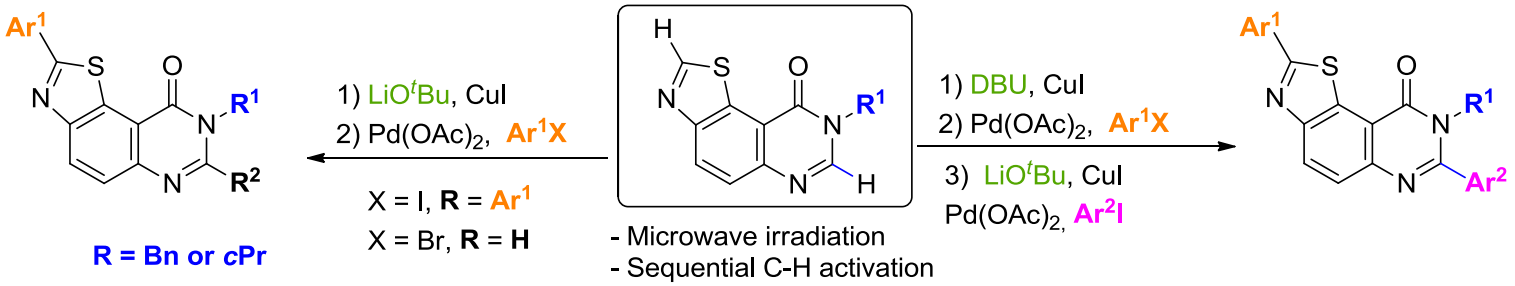
Driven by the need of structural modification to establish structure–activity relationships, a selective functionalization of the thiazolo[5,4-*f*]quinazolin-9(8*H*)-one scaffold has been envisioned through sequential activation of C-H bonds to furnish diarylated compounds .

[1] Chaikuad, A.; Diharce, J.; Schröder, M.; Foucourt, A.; Leblond, B.; Casagrande, A.-S.; Désiré, L.; Bonnet, P.; Knapp, S.; Besson, T. *J. Med. Chem.* **2016**, *59,* 10315.

[2] Hédou, D.; Godeau, J.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 578; (b) Hédou, D.; Dubouilh-Benard, C.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 794.

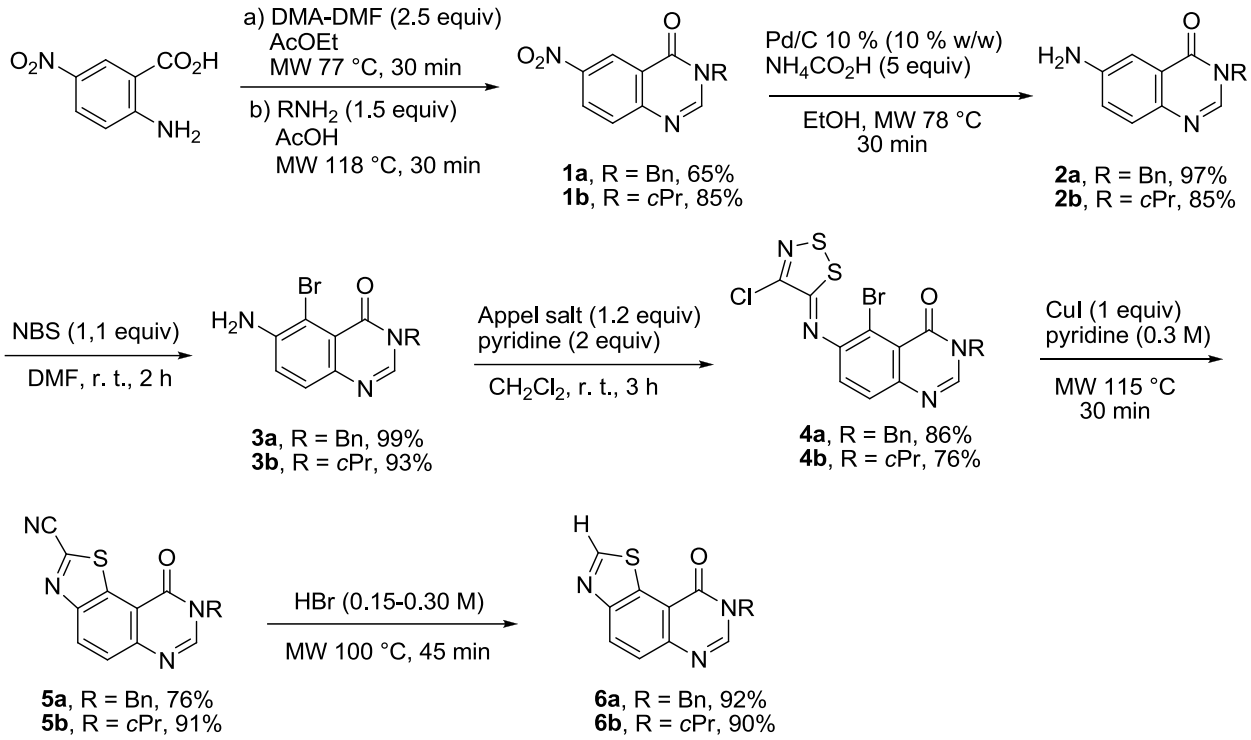
Results and discussion

C-C bond formation through a C-H bond activation emerged as a powerful tool for the late-stage diversification of these valuable scaffolds.



Differently substituted N^8-substituted-2,7-diaryl-thiazoloquinazolin-9(8*H*)-ones were envisioned via regioselective C-H bond activation of thiazolo[5,4-*f*]quinazolin-9(8*H*)-one backbone in the hope to furnish the corresponding C2 and C7-arylated expected scaffold.

Results and discussion : Synthesis of N^8-benzyl-thiazolo[5,4-*f*]quinazolin-9(8*H*)-one **6a** and N^8-cyclopropyl-thiazolo[5,4-*f*]quinazolin-9(8*H*)-one **6b** starting from 5-nitro anthranilic acid

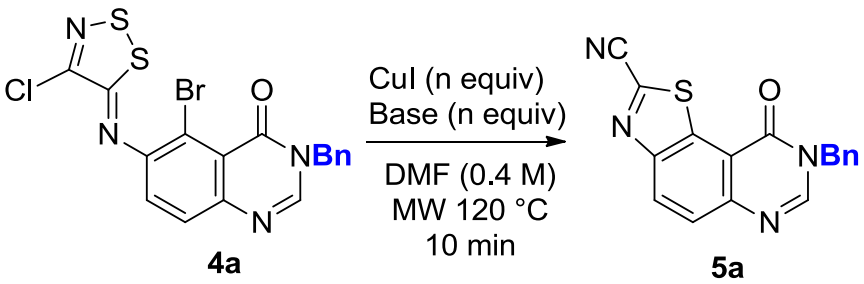


The synthesis of thiazolo[5,4-*f*]quinazolin-9(8*H*)-one-2-carbonitriles **5a** and **5b** can be performed in 5 steps starting from commercially available 5-nitro-anthranilic acid. The key steps are the reaction of the aniline derivative **3** with 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt) following by the cyclisation of the intermediate imine **4**. Access to compounds **6a-b** was finally performed by heating the corresponding precursor **5a-b** in HBr. Nevertheless previous studies on C-H arylation of compound **5a**, showing that treatment of this compound with 2 equivalents of *t*BuOLi and 50 mol% of CuI for 10 min afforded small quantities of product **6a**, allowed us to modify the synthetic route.

Results and discussion Effect of the additives on the synthesis of thiazolo[5,4-*f*]quinazolin-9(8*H*)-one-2-carbonitrile **5a** from **4a**

We reasoned that compound **6a** could be obtained from intermediate **4a** in a one-pot cyclisation-decyanation process in the presence of *t*BuOLi, instead of the most toxic pyridine and an excess of CuI. No decyanation reaction was observed under these conditions but we were pleased to find that compound **5a** was isolated in 70% yield (Table, entry 1). Increasing the amount of the base gave 9% of **5a** besides traces of decyanated product **6a** but led mainly to the degradation of compound **4a** (entry 5). Longer reaction time or decreasing amount of *t*BuOLi did not affect the cyclisation step (entries 6-8). More surprisingly, compound **5a** was obtained without *t*BuOLi but in lower yield even with longer reaction time (entries 9 and 10).

entry	CuI (n equiv)	Base (n equiv)	5a (%)[a]
1	1.5	tBuOLi (2)	70
2[b]	1.5	tBuOLi (2)	45
3	-	tBuOLi (2)	72
4	1.5	-	42
5	-	tBuOLi (3)	9
6[b]	-	tBuOLi (2)	73
7	-	tBuOLi (1)	71
8	-	tBuOLi (0.5)	61
9	-	-	15
10[c]	-	-	30
11	-	DBU (2)	51



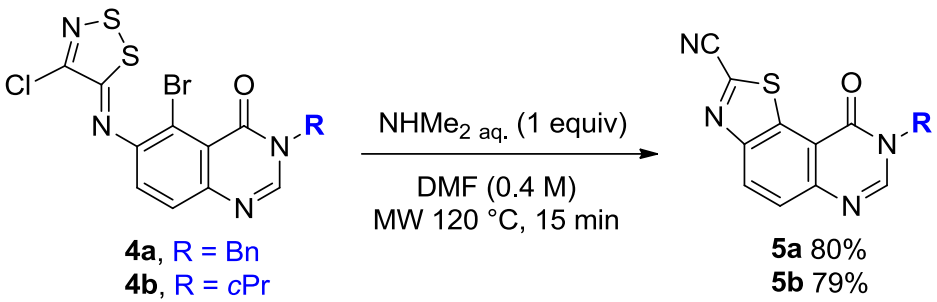
[a] Yield of isolated compound. [b] Reaction time is 1 h. [c] Reaction time is 5 h.

Results and discussion : Metal-free synthesis of thiazolo[5,4-*f*]quinazolin-9(8*H*)-one-2-carbonitriles **5a-b** from **4a-b**

We speculated that the rapid microwave decomposition of DMF leading to generated *in situ* dimethylamine might render compound **4a** more likely to cyclize. In fact, the results observed in entries 8 and 9 are consistent with the recent reported studies that emphasis highest decomposition rate of DMF in the presence of a base such as tert-Butylate. With regard to the effect of additives on the decomposition of DMF, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and ammonium hydroxide solution were tested.

entry	CuI (n equiv)	Base (n equiv)	5a (%)[a]
12	-	NH_4OH (2)	59
13	-	$NHMe_2$ (2)	67
14	-	$NHMe_2$ (1)	76
15[d]	-	$NHMe_2$ (1)	64
16[e]	-	$NHMe_2$ (1)	80
17[f]	-	$NHMe_2$ (1)	78

[d] Reaction time is 5 min. [e] Reaction time is 15 min. [f] Reaction time is 30 min.



4a, R = Bn
4b, R = *c*Pr

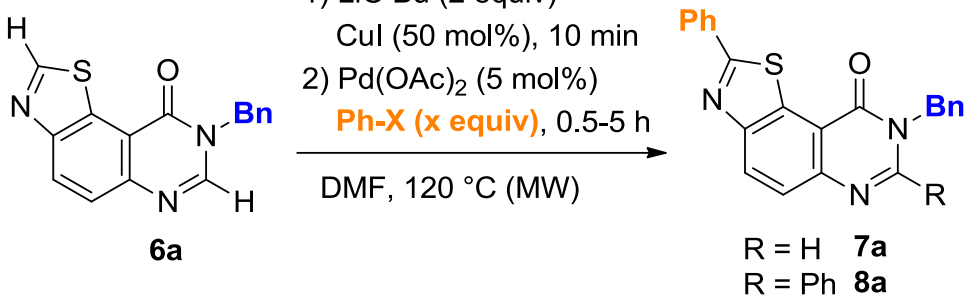
5a 80%
5b 79%

We were delighted to find that when compound **4a** was treated with an aqueous solution of dimethylamine (entries 13-17) the tricyclic core **5a** was obtained in up to 80% yield (entry 16). It could be noticed that DMF-free reaction led to a mixture of starting material **4a**, compound **5a** and degradation. Applying the optimized condition reaction to compound **4b** gave the tricyclic product **5b** in 79% yield (Scheme).

Results and discussion : Arylation of N^8-Benzylated-Thiazolo[5,4-*f*]quinazolin-9(8*H*)-one **6a** With Aryl Halides[a]

We opted to use the N^8-benzylated-thiazolo-quinazolin-9(8*H*)-one **6a** as the substrate. Under previous conditions, a mixture of C2-mono- and C2/C7-bis-phenylated products **7a** and **8a**, was obtained among starting material **6a**. However, when the loading of aryl iodide was increased and reaction time was prolonged to 5 h, diphenylated product **8a** was interestingly obtained in up to 68% yield (entry 6).

entry	time (h)	PhX (x equiv)	7a (yield %)[b]	8a (yield %)[b]
1	0,5	PhI (2)	27	45 (23) [c]
2 [d]	1	PhI (2)	30	0 (64) [c]
3	4	PhI (2)	28	53 (0) [c]
4	4	PhI (3)	32	59 (0) [c]
5 [e]	4	PhI (3)	31	62 (0) [c]
6	**5**	**PhI (4)**	**27**	**68 (0) [c]**
7	3	PhBr (2)	93	0 (0) [c]
8	3	PhCl (2)	0	0 (100) [c]

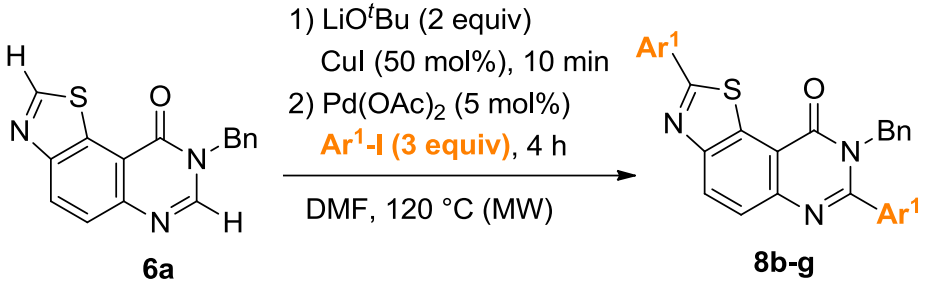


[a] Conditions: Reactions were performed in a sealed tube at 0.4 M with premixing CuI (50 mol%), LiO*t*Bu (2 equiv) and **6a** (1 equiv) in a microwave reactor, before adding PhX (x equiv), $Pd(OAc)_2$ (5 mol%). [b] Reported yields are isolated yields. [c] yields of recovered starting material **6a**. [d] The reaction was performed without CuI. [e] the reaction was performed with 1 equiv of CuI.

Results and discussion : Scope of Bis-arylation Reactions [a]

With acceptable conditions established, we explored the scope of the bis-arylation reaction (Table) with various aryl iodides. When aryl iodide was introduced, the resulting 2-aryl-thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones were reactive enough to perform a second arylation, yielding significant amounts of 2,7 -homodiarylated products **8b-f**. This double C2/C7 arylation protocol yielded targeted compounds in moderate yields. Whatever the applied reaction conditions, the bis-arylation was not complete.

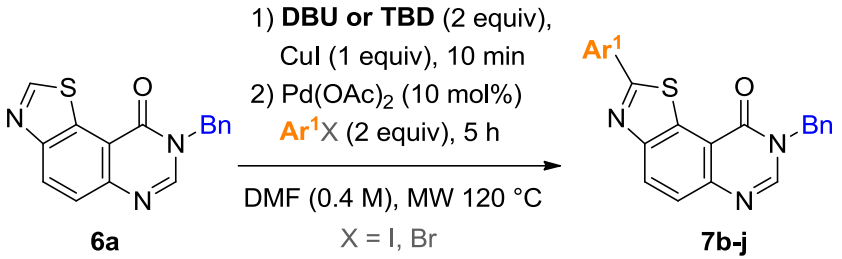
entry	Ar-	compd	yield (%)[b]
1	4-Me-Ph-	**8b**	63 (30)[c]
2	4-MeO-Ph-	**8c**	67 (29)[c]
3	4-Cl-Ph-	**8d**	56 (41)[c]
4	4-F-Ph-	**8e**	26 (58)[c]
5	4-CN-Ph-	**8f**	55 (38)[c]
6	2,4-Cl-Ph-	**8g**	0 (88)[c]



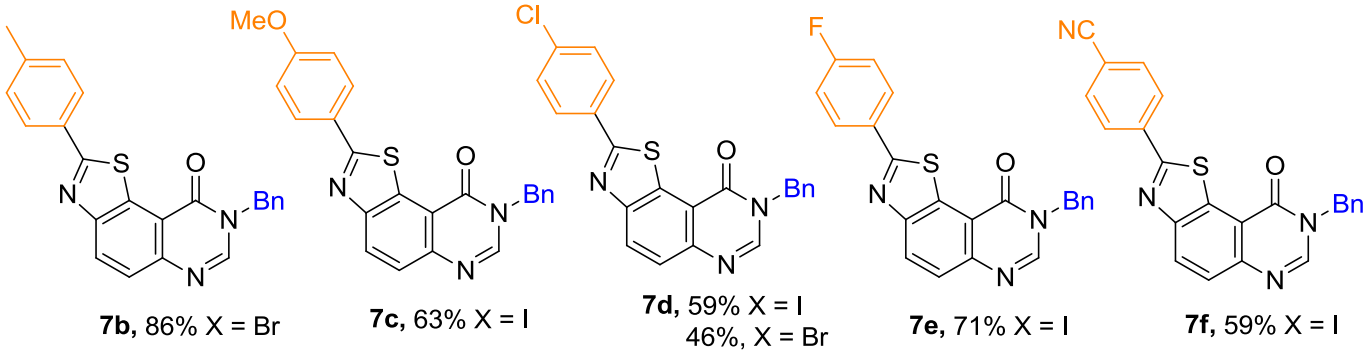
6a **8b-g**

[a] Conditions: Reactions were performed in a sealed tube at 0.4 M with premixing **6a** (1 equiv), LiO^{t}Bu (2 equiv), and CuI (50 mol %) in a microwave reactor, before adding ArI (3 equiv), Pd(OAc)$_2$ (5 mol %). [b] Reported yields are isolated yields. [c] isolated yields of corresponding compound **6b-g**.

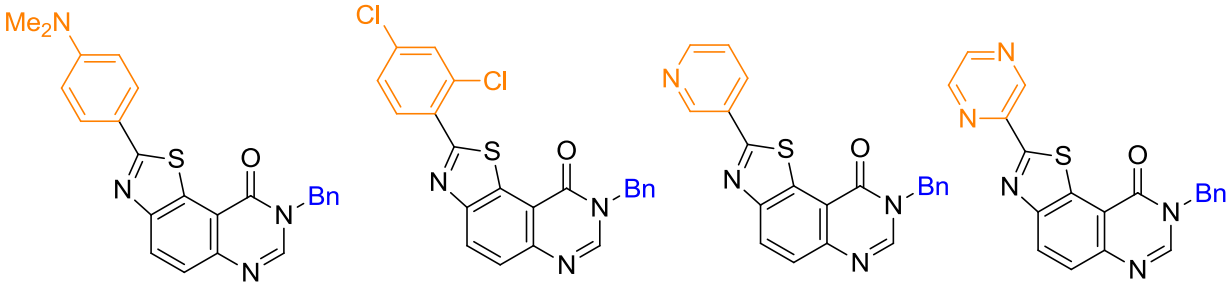
Results and discussion : Scope of the C2 Arylation of N^8-Benzylated-Thiazolo[5,4-*f*]quinazolin-9(8*H*)-one 1 with Aryl Halides[a]



6a **7b-j**



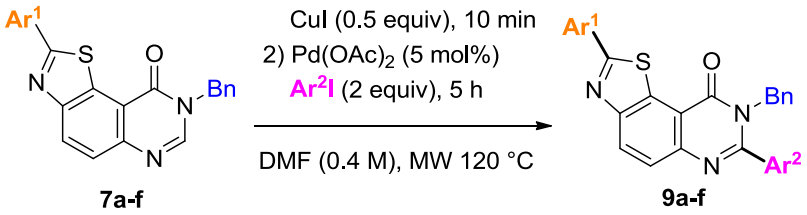
7b, 86% X = Br **7c,** 63% X = I **7d,** 59% X = I, 46%, X = Br **7e,** 71% X = I **7f,** 59% X = I

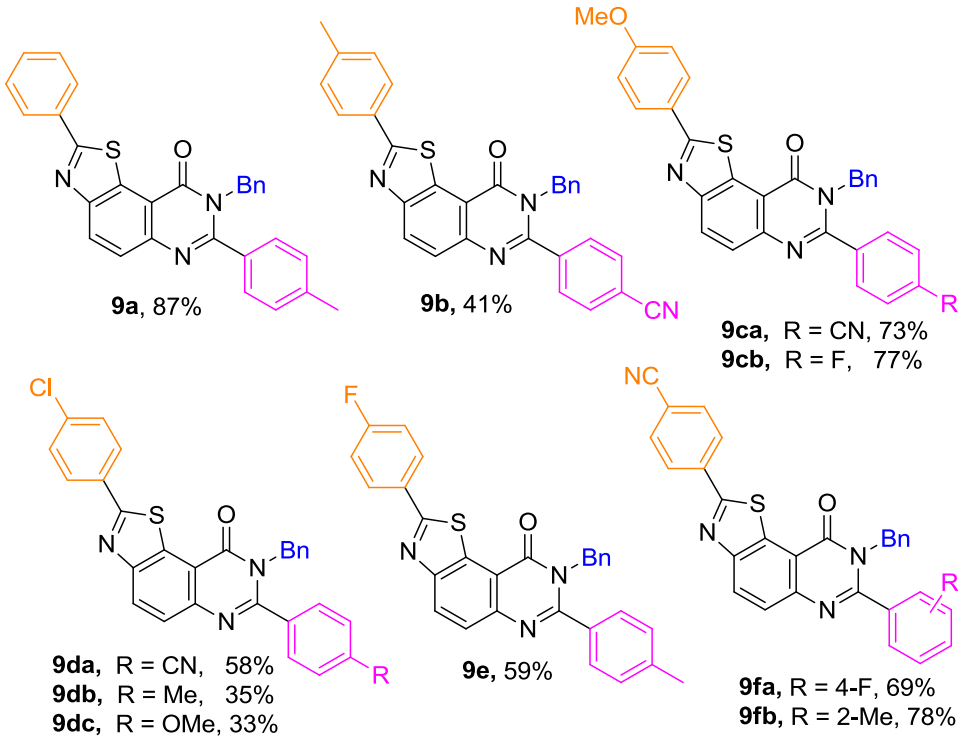


7h, 87% X = Br **7g,** 62% X = I **7i,** 69% X = I **7j,** 47% X = I

[a] premixing **6a** (1 equiv), DBU (2 equiv) and CuI (1 equiv), before adding ArI or ArBr (2 equiv), $Pd(OAc)_2$ (10 mol%) for 5 h.

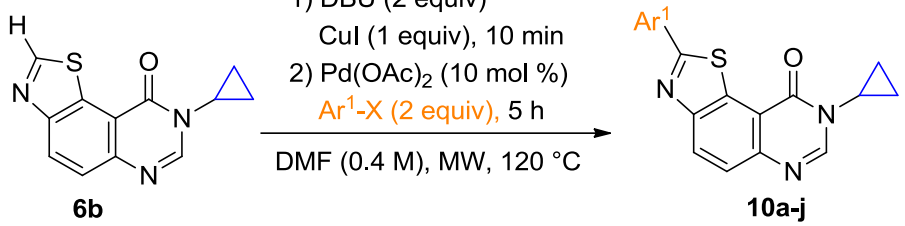
Results and discussion : Regioselective C7 Arylation of 2-Aryl-N^3-Benzylated-Thiazolo[5,4-*f*] quinazolin-9(8*H*)-ones 2a-f [a]

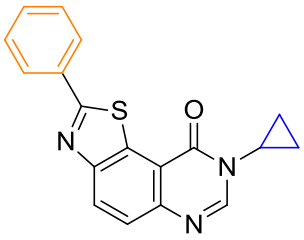




[a] premixing **2** (1 equiv), LiO*t*Bu (2 equiv) and CuI (50 mol %), before adding ArI (2 equiv), $Pd(OAc)_2$ (5 mol %) for 5 h.

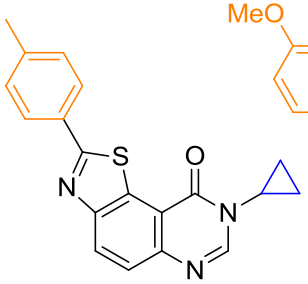
Results and discussion : Scope of the C2 Arylation of N^8-cyclopropyl-Thiazolo[5,4-*f*]quinazolin-9(8*H*)-one 1 with Aryl Halides[a]



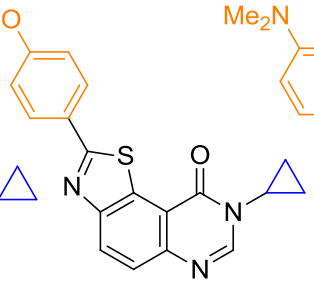


10a

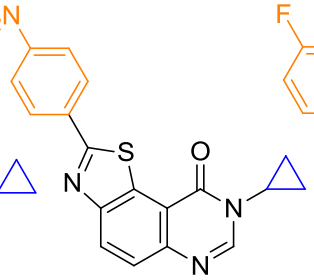
*t*BuOLi, X= Br 19%
DBU, X = I 58%



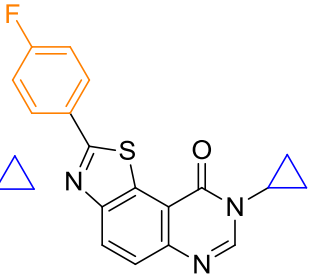
10b, 64% X = I



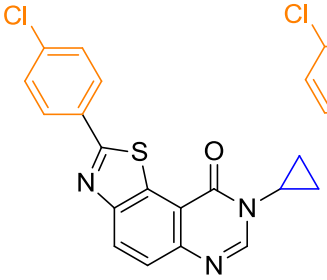
10c, 76% X = I



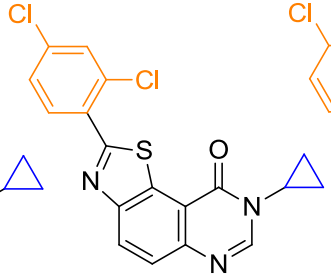
10d, 31% X = Br



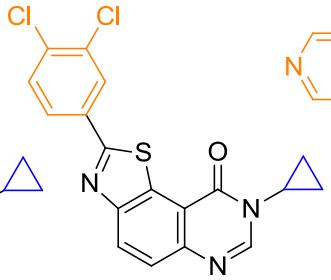
10e, 52% X = I



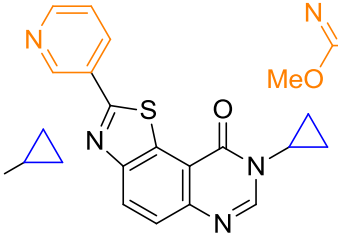
10f, 67% X = I



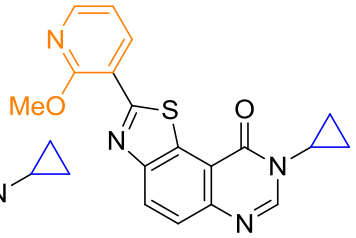
10g, 65% X = I



10h, 69% X = I



10i, 43% X = I[b]



10j, 33% X = I

[a] Premixing **6b** (1 equiv), DBU (2 equiv) and CuI (1 equiv) at 120 °C for 10 min, before adding $Pd(OAc)_2$ (10 mol%) and ArI or ArBr (2 equiv) for 5 h. [b] 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD) was used as base instead of DBU.

Results and discussion : Kinase inhibitory activity[a,b,c] of the thiazolo[5,4-*f*]quinazoline described :

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.

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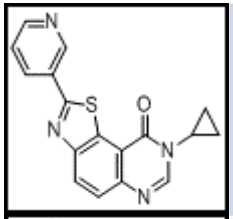
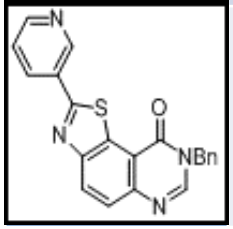
Results and discussion

Kinase inhibitory activity[a,b,c] of the thiazolo[5,4-*f*]quinazoline described : Only two monoarylated compounds show high inhibition values for kinase involved in Alzheimer's disease.

		CLK1	CLK2	CLK3	CLK4	DYRK1A	DYRK1B	DYRK2	DYRK3	GSK3
10i		**0.018**	0.083	5.3	0.035	**0.011**	0.120	**0.013**	0.023	0.068
7i		2	4	>10	0.630	**0.012**	0.110	**0.001**	<0.001	3.7

[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%.

Harmine is a β-carboline alkaloid known to be a potent inhibitor of DYRK1A (IC_{50} = 0,029 µM in the conditions tested).

Conclusions

According to the need of structural modification to establish structure–activity relationships, we described efficient methods for the late-stage functionalization of N^8-benzyl and N^8-cyclopropyl thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones. Both procedures tolerate a large panel of substituents on the aryl halide. A metal-free synthesis of the precursors was also described. Two mono-arylated compounds show high inhibition values for kinase involved in Alzheimer's disease.

Acknowledgments

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