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Conception of DYRK1A Kinase Inhibitors via Metal-Catalyzed C–H Arylation, inspired by Fragment-Growing Studies.

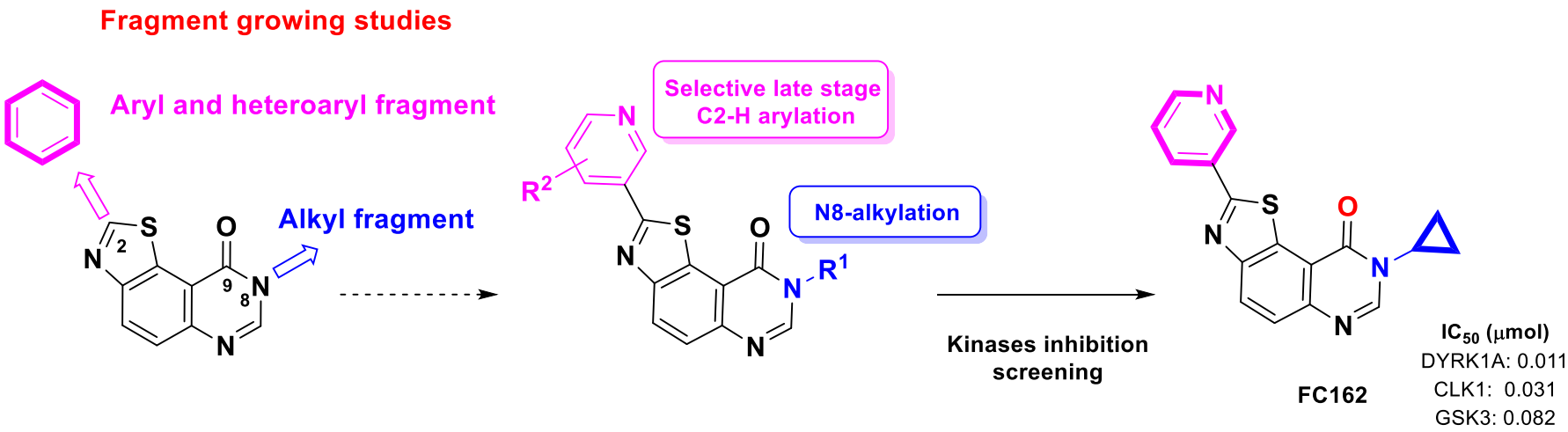
Florence Couly [1], Julien Diharce [2], Pascal Bonnet [2], Laurent Meijer [3], Corinne Fruit[1,*] and Thierry Besson[1,*]

[1] Normandie Univ, UNIROUEN, INSA Rouen, CNRS, COBRA UMR 6014, 76000 Rouen, France
[2] Université d'Orléans, ICOA, UMR CNRS 7311, BP 6759, 45067 Orléans Cedex 2, France
[3] ManRos Therapeutics, Perharidy Peninsula, 29680 Roscoff, France

* Corresponding authors: corinne.fruit@univ-rouen.fr; thierry.besson@univ-rouen.fr

Conception of DYRK1A Kinase Inhibitors via Metal-Catalyzed C–H Arylation inspired by Fragment-Growing Studies

Graphical Abstract



Abstract:

Efficient metal catalyzed C–H arylation of 8-alkyl-thiazolo[5,4-*f*]-quinazolin-9-ones was explored for SAR studies. Application of this powerful chemical tool at the last stage of the synthesis of kinase inhibitors allowed the synthesis of arrays of molecules inspired by fragment-growing studies generated by molecular modeling calculations. Among the potentially active compounds designed through this strategy, **FC162** (**Cc**) exhibits nanomolar IC_{50} values against some kinases, and is the best candidate for development as a DYRK kinase inhibitor.

Keywords: thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones; microwave-assisted synthesis; C–H arylation; protein kinases; DYRK1A; CDK5; GSK-3; CLK1; CK1

Introduction

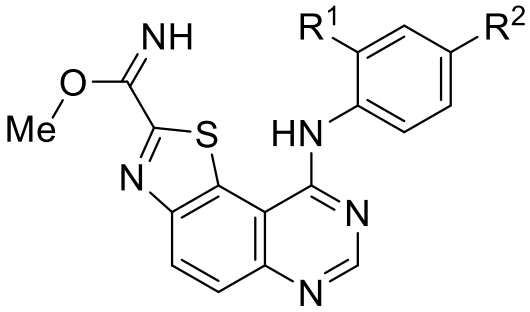
Kinases catalyse protein phosphorylation, a key cellular regulatory mechanism, which is frequently dysregulated in human diseases. Protein kinases have consequently been linked to the progress of a variety of diseases including cancer and neurodegenerative disorders. Therefore, the search for therapeutic inhibitors of specific kinases has been developed in the last three decades as a major approach to discover new drugs [1,2].

Our group is focused on the regulation of dual-specificity tyrosine phosphorylation-regulated kinase 1A (**DYRK1A**), a conserved eukaryotic kinase that belongs to the DYRK family and the CMGC group, which includes cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAP kinases), glycogen synthase kinases (GSK), and Ccd2-like kinases (CLKs) [3]

(1) Martin, L.; Latypova, X.; Wilson, C.M.; Magnaudeix, A.; Perrin, M.-L.; Terro, F. *Ageing Res. Rev.* **2013**, *12*, 289–309. doi:10.1016/j.arr.2012.06.003.
(2) Weinmann, H.; Metternich, R. *Chembiochem* **2005**, *6*, 455–459. doi:10.1002/cbic.200500034.
(3) Varjosalo, M.; Keskitalo, S.; Van Drogen, A.; Nurkkala, H.; Vichalkovski, A.; Aebersold, R.; Gstaiger, M. *Cell Rep.* **2013**, *3*, 1306–1320. doi: 10.1016/j.celrep.2013.03.027

Introduction

Five years ago, a series of tricyclic aminopyrimidine derivatives was synthesized and evaluated on DYRK1A and DYRK1B. Five derivatives (**EHT series**) displayed single-digit nanomolar or subnanomolar IC_{50} values, and were quite specific towards the CMGC group [5,6].



I - V

Compound	R^1	R^2	DYRK1A / 1B*
I (EHT 5372)	Cl	Cl	0.22 / 0.28
II (EHT 1610	F	OMe	0.36 / 0.59
III (EHT 9851)	F	F	0.94 / 1.07
IV (EHT 6840)	F	Cl	0.99 / 1.63
V (EHT 3556)	H	Me	0.98 / 2.83

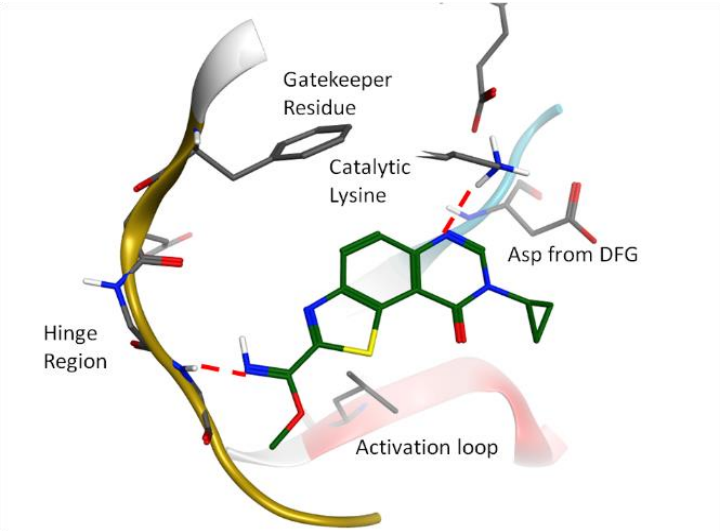
* IC_{50} (nM)

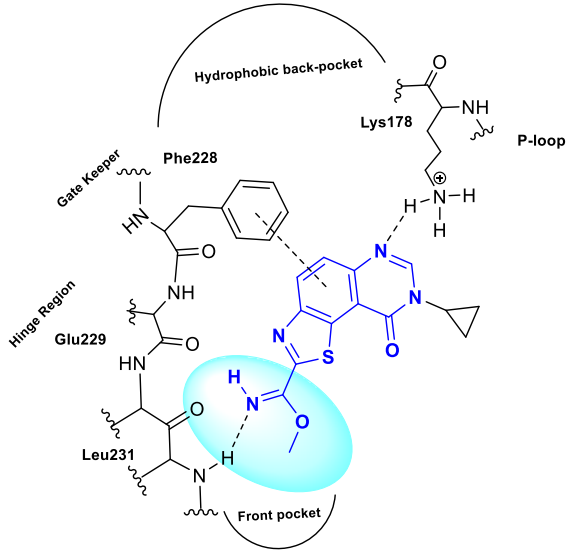
(5) Leblond, B.; Casagrande, A.-S.; Désiré, L.; Foucourt A.; Besson, T. DYRK1 inhibitors and uses thereof WO 2013026806.

(6) Coutadeur, S.; Benyamine, H.; Delalonde, L.; de Oliveira, C.; Leblond, B.; Foucourt, A.; Besson, T. ; Casagrande, A.-S.; Taverne, T. ; Girard, A. ; Pando, M.P.; Désiré, L. *J. Neurochem.* **2015**, *133*, 440–451. doi:10.1111/jnc.13018.

Introduction

Based on the results obtained with the crystal structure of DYRK2 in complex with **EHT 5372** and **EHT 1610** products, docking experiments and calculations were performed, and resulting models suggested that 9-oxo-inhibitors displayed binding modes identical to that of their 9-amino-congeners.

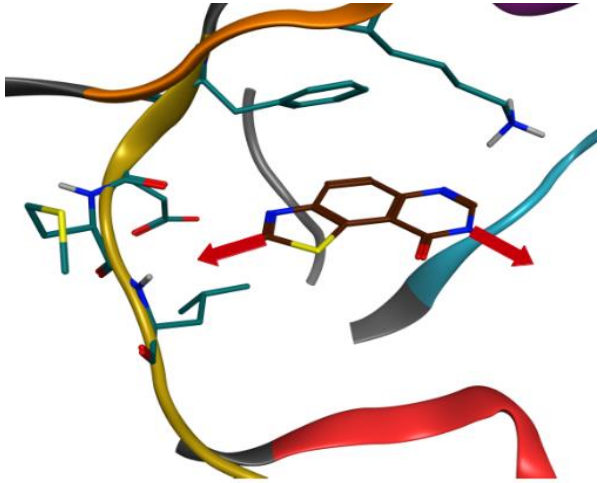




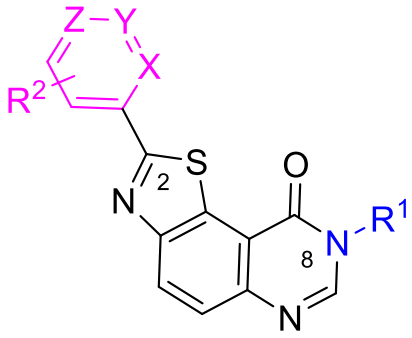
(7) Chaikuad, A.; Diharce, J.; Schröder, M.; Foucourt, A.; Leblond, B.; Casagrande, A.-S.; Désiré, L.; Bonnet, P.; Knapp, S.; Besson, T. *J. Med. Chem.* **2016**, *59*, 10315–10321. doi:10.1021/acs.jmedchem.6b01083.

Introduction

A fragment-growing approach was performed using a novel *in silico* tool that drills down through, to evaluate hundreds of thousands fragments extracted from co-crystallized kinase/inhibitor complexes. Addition of aromatic fragments on C2 seemed to increase the interaction with the hinge region. A library of novel C2-arylated N8-alkyl thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones was envisioned by addition of (hetero)-aromatic fragments.



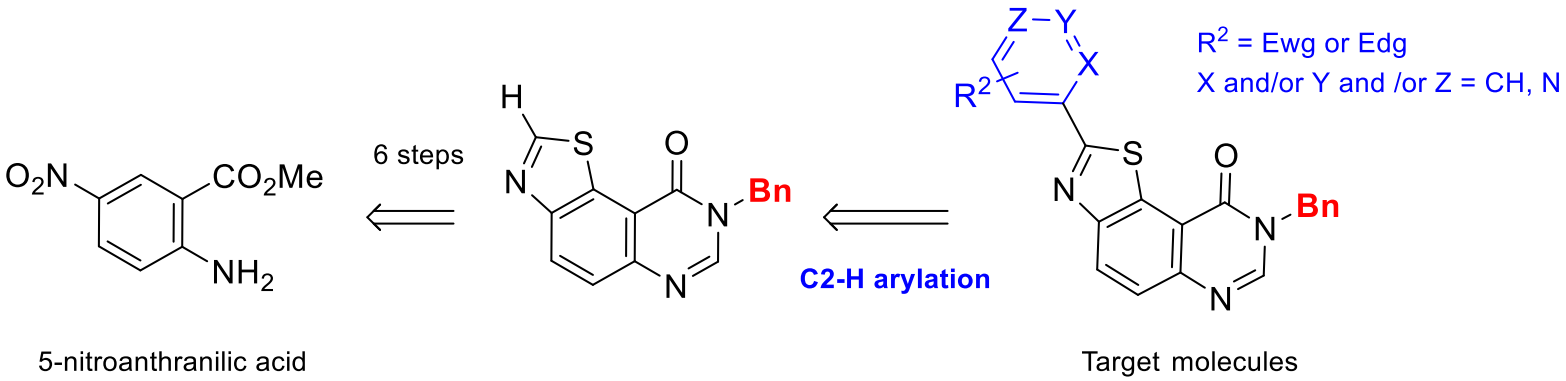
In silico fragment-growing calculations



R^1 = Alkyl
R^2 = Ewg or Edg
X and/or Y and /or Z = CH, N

Introduction

As a result in our recent experience in carbon–carbon bond formation [8-10], a regioselective C–H bond activation was performed to provide corresponding C2-arylated valuable compounds. Most of the syntheses were achieved under microwave irradiation as a powerful alternative to traditional heating with economic and environmental benefits.



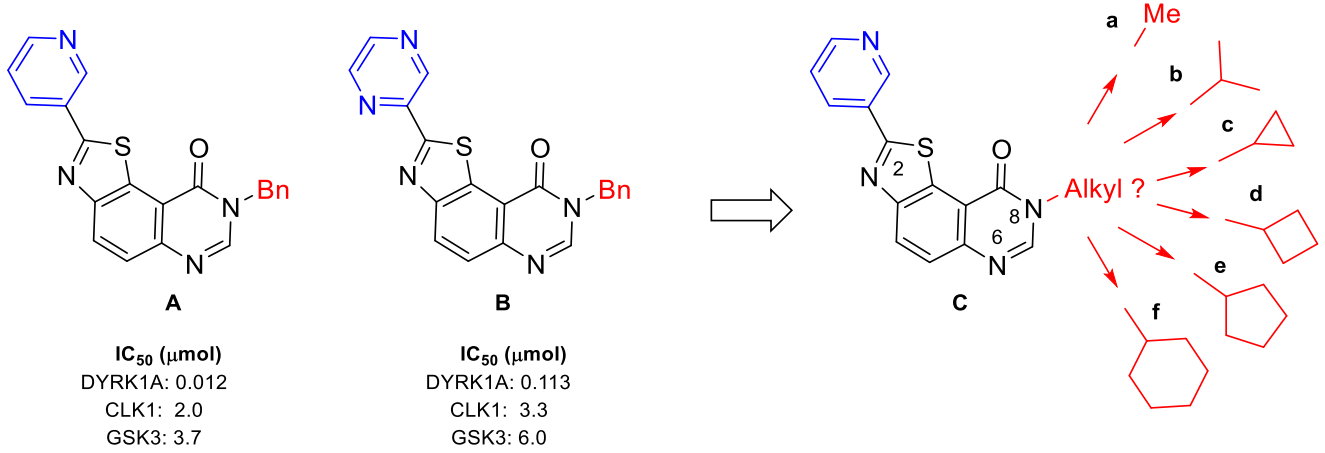
(8) Harari, M.; Couly, F.; Fruit, C.; Besson, T. *Org. Lett.* **2016**, *18*, 3282–3285. doi:10.1021/acs.orglett.6b01552.
(9) Couly, F.; Dubouilh-Benard, C.; Besson, T.; Fruit, C. *Synthesis* **2017**, *49*, 4615–4622. doi:10.1055/s-0036-1588434.
(10) Besson, T.; Fruit, C. *Synthesis* **2016**, *48*, 3879–3889. doi:10.1039/C6OB00227G.

Results and discussion

The inhibitory potency of N8-benzylated thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones obtained was evaluated according to standard methods [11,12] on a panel of kinases (for details see kinase profiling paragraph).

Among the tested molecules, only two (**A** and **B**) exhibited micromolar IC_{50} values against kinases CLK1 and GSK3, and nanomolar range inhibition against DYRK1A . Compound **A** was the most active.

Taking these preliminary results into account, a new series **C** was designed by keeping the 3-pyridinyl moiety in position C2, and modifying the alkyl substituents in position N8 of the thiazolo[5,4-*f*]quinazolin-9(8*H*)-ones.



A

IC_{50} (μmol)
DYRK1A: 0.012
CLK1: 2.0
GSK3: 3.7

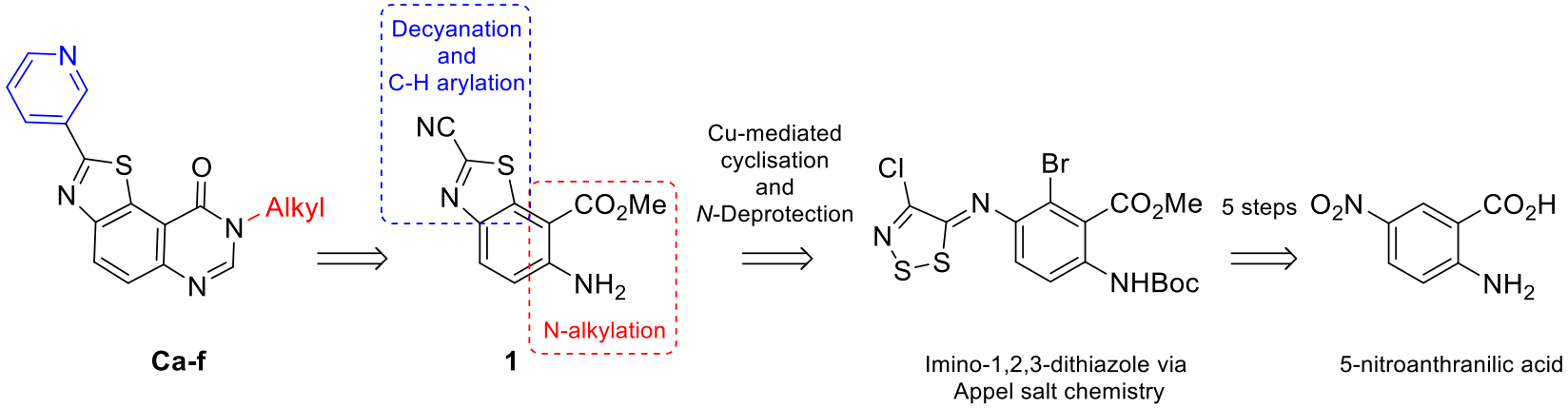
B

IC_{50} (μmol)
DYRK1A: 0.113
CLK1: 3.3
GSK3: 6.0

(11) Hédou, D.; Godeau, J.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 578. doi:10.3390/molecules21050578.
(12) Hédou, D.; Dubouilh-Benard, C.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 794. doi:10.3390/molecules21060794.

Results and discussion

Retrosynthetic route of series **C** products using compound **1** as intermediate.

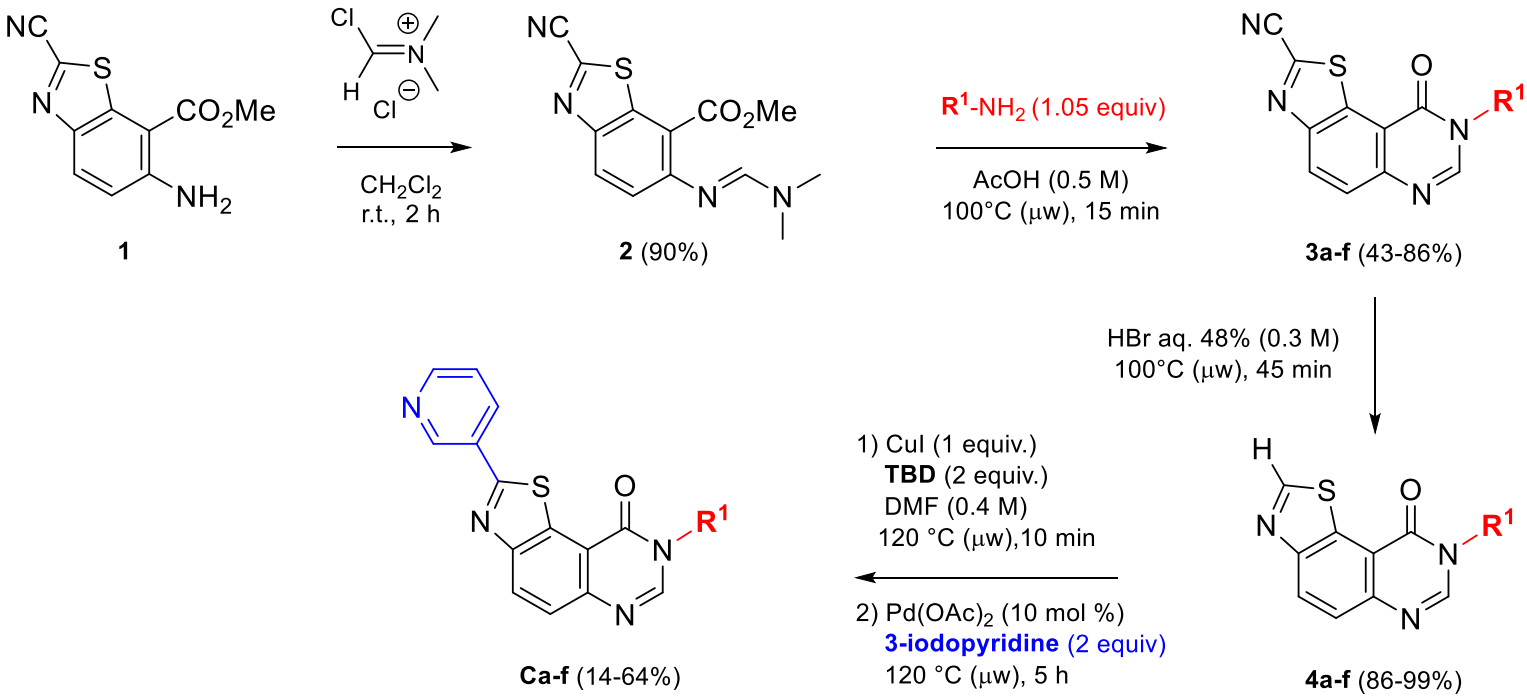


The target molecules (series **C**) were synthesized via the polyfunctionalized methyl 6-amino-2-cyanobenzo[d]thiazole-7-carboxylate (**1**) [13]. Here, again, the key step in the synthesis of **1** involves the sulfur-rich Appel's salt, and cyclization of the intermediate imino-1,2,3-dithiazole which was transformed into the target benzothiazole. In this pathway, the pyrimidinone part was formed at the last stage of the synthesis.

(13) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2018**, **23**, 2181. DOI : 10.3390/molecules23092181

Results and discussion

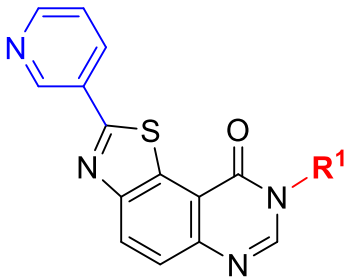
Synthesis of series **3a–f**, **4a–f**, and **Ca–f**.



The second step consisted in substituting 2-(pyridin-3-yl)thiazolo[5,4-*f*]quinazolin-9(8*H*)-one with alkyl groups, such as methyl, *iso*-propyl, or cycloakyl containing at least 4 carbons. The last step concerns C2-H arylation of the tricyclic core.

(14) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2018**, *23*, 2181. DOI : 10.3390/molecules23092181

Results and discussion



Ca-f

Table 2. Chemical structures (R^1) and yields obtained for the synthesis of series **3a–f**, **4a–f**, and **Ca–f**.

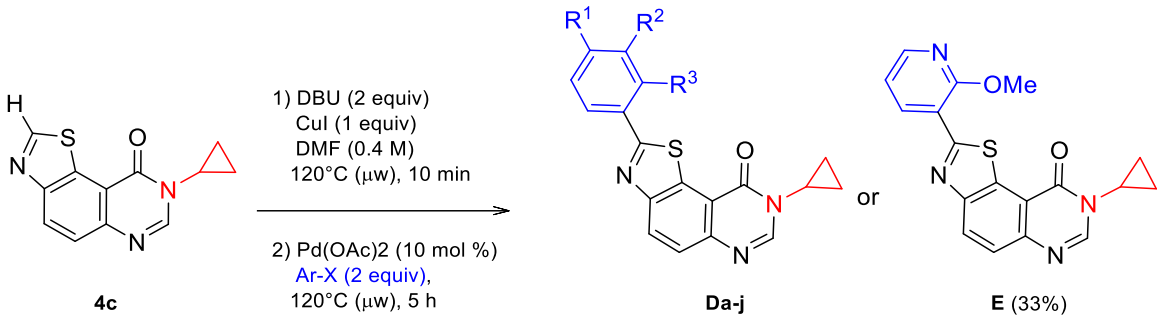
–R^1	Compound	Yield [a] (%)	Compound	Yield [a] (%)	Compound	Yield [a] (%)
Me	**3a**	70	**4a**	98	**Ca**	14
	3b	43	**4b**	99	**Cb**	64
	3c	86	**4c**	86	**Cc**	43
	3d	65	**4d**	97	**Cd**	57
	3e	60	**4e**	98	**Ce**	55
	3f	50	**4f**	98	**Cf**	54

[a] Isolated yield.

(14) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2018**, *23*, 2181. DOI : 10.3390/molecules23092181

Results and discussion

Synthetic route to series **Da–j** and compound **E,** for completion of SAR studies



4c **Da-j** **E** (33%)

Product D	R^1	R^2	R^3	X(Ar)	Yield [b] (%)
a	H	H	H	Br	58
b	Me	H	H	Br	64
c	MeO	H	H	I	76
d	Cl	H	H	I	67
e	F	H	H	I	52
f	CN	H	H	I	-[c]
h	NMe_2	H	H	Br	31
i	Cl	H	Cl	I	65
j	Cl	Cl	H	I	69

Chemical structures and yields obtained for the synthesis [a] of series **Da–j** (R^1, R^2, and R^3).
[a] Premixing **4c**, DBU, and CuI, 10 min before adding ArI or ArBr, $Pd(OAc)_2$ and stirring for 5 h;
[b] Isolated yields; [c] Not obtained.

Results and discussion

Kinase inhibitory activity [a,b,c] of the thiazolo[5,4-*f*]quinazoline derivatives (**A**, **B**, **Ca-j**, **Da-j** and **E**)

Results demonstrate that the thiazolo[5,4-*f*]quinazolin-9(8*H*)-one **Cc** also called **FC162**, shows significant inhibitory activity against a set of five kinases.

Compounds	CDK5/p25	CK1δ/ε	CLK1	DYRK1A	GSK-3α/β
A	>10	>10	2.0	0,012	3.7
B	>10	>10	3.33	0.133	6.0
Cc (FC162)	n.t.	6.0	0.018	0.011	0.068
Ca, Cb, Cd-f	n.t.	>10	>10	>10	>10
Da–i	>10	>10	>10	>10	≥10
E	>10	>10	>10	>10	≥10
Harmine	>10	1.5	0,026	0,029	>10

[a] IC_{50} values are reported in µM. The most significant results are presented in bold; [b] Kinase activities were assayed in triplicate; [c] Typically, the standard deviation of single data points was below 10%; [d] n.t., not tested.

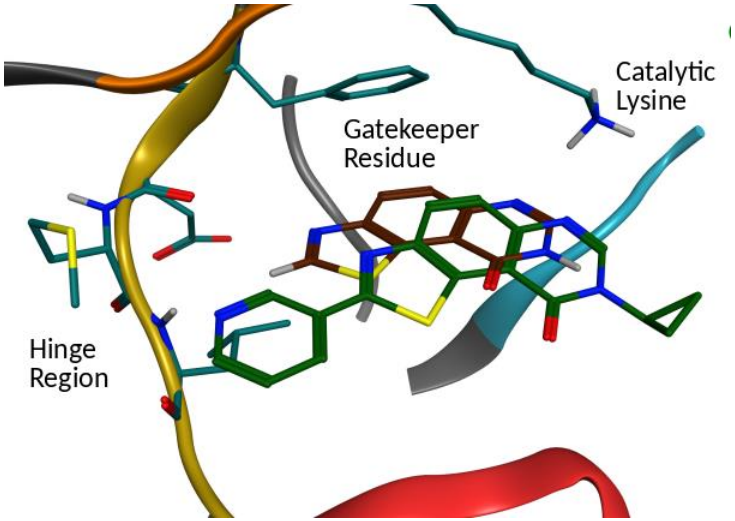
All compounds were first tested at a final concentration of 10 µM. Compounds showing less than 50% inhibition were considered as inactive (IC_{50} >10 µM). Compounds displaying more than 50% inhibition at 10 µM were next tested over a wide range of concentrations (usually from 0.01 to 10 µM), and IC_{50} values were determined from the dose–response curves (Sigma-Plot). Harmine, a β-carboline alkaloid known to inhibit DYRK1A , was used as a positive control.

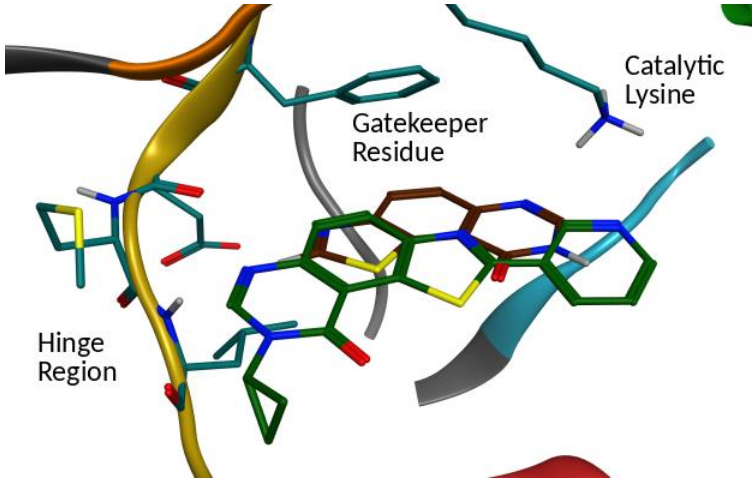
(13) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2018**, *23*, 2181. DOI : 10.3390/molecules23092181

Results and discussion

Docking calculations were next performed in order to predict the molecular interactions of **FC162** with DYRK1A. Two main binding modes were obtained. **Left**, first predicted binding mode (green), with the same orientation of the skeleton, but slightly shifted. **Right**, second predicted binding mode, in which the skeleton is flipped compared to its initial placement (in brown).

The docking score of the two poses was quite similar, thus, both binding modes are equally possible for this compound.

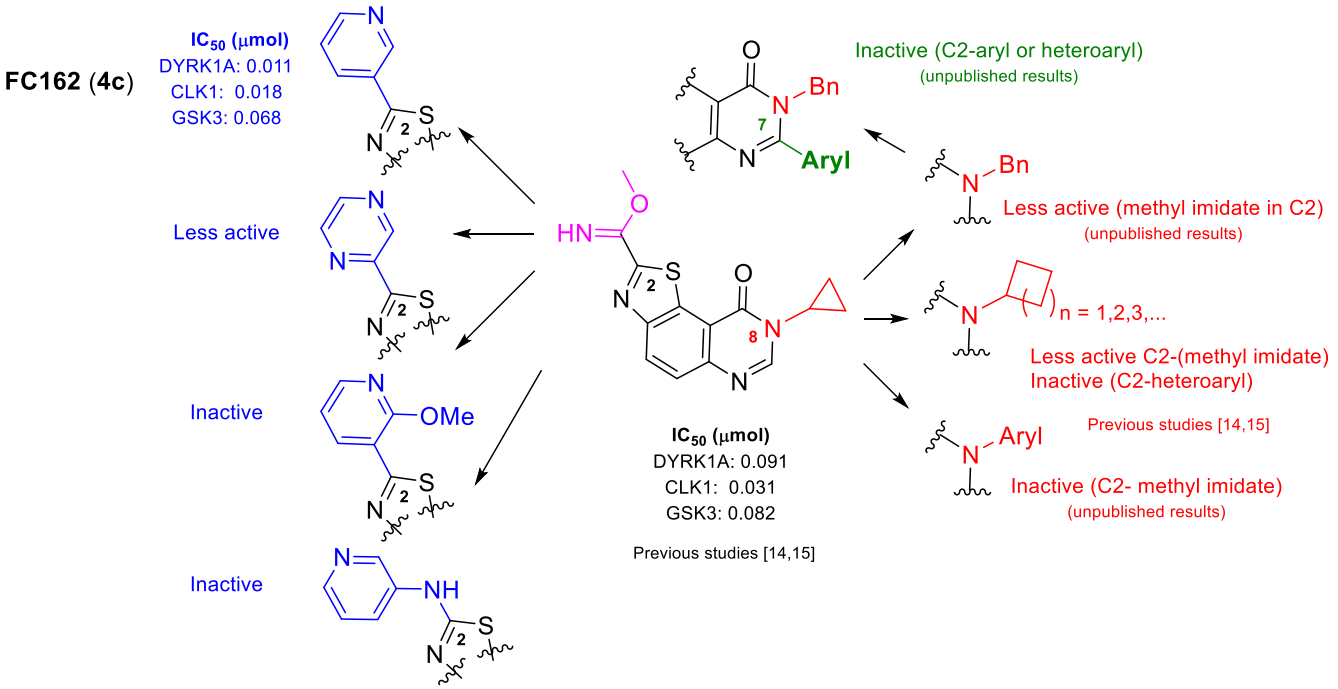




(13) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2018**, *23*, 2181. DOI : 10.3390/molecules23092181

Results and discussion

The SAR study revealed that **FC162** with a 3-pyridinyl group in position 2 had a higher activity than the series of phenylated derivatives **A** and **B**. These results are notably in agreement with the fragment-growing experiments, which suggested replacement of the imidate group by a more stable heteroaromatic substituent.



(14) Hédou, D.; Godeau, J.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 578. doi:10.3390/molecules21050578.
(15) Hédou, D.; Dubouilh-Benard, C.; Loaëc, N.; Meijer, L.; Fruit, C.; Besson, T. *Molecules* **2016**, *21*, 794. doi:10.3390/molecules21060794.

Conclusions

This work demonstrates the efficacy of synthetic methodologies, such as C–H arylation of arenes and hetero-arenes for SAR studies. The application of this powerful tool at the last stage of the synthesis of kinase inhibitors allowed the synthesis of arrays of molecules inspired by fragment-growing studies generated by molecular modeling calculations. Among the potential active compounds generated through this strategy, **FC162** (**Cc**) was found to be the best candidate for development as a DYRK inhibitor.

Acknowledgments

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