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Synthesis and biological evaluation of linear thiazolo[4,5-*g*] and [5,4-*g*] quinazolines, analogues of V-shaped DYRK1A inhibitors EHT1610 and FC162

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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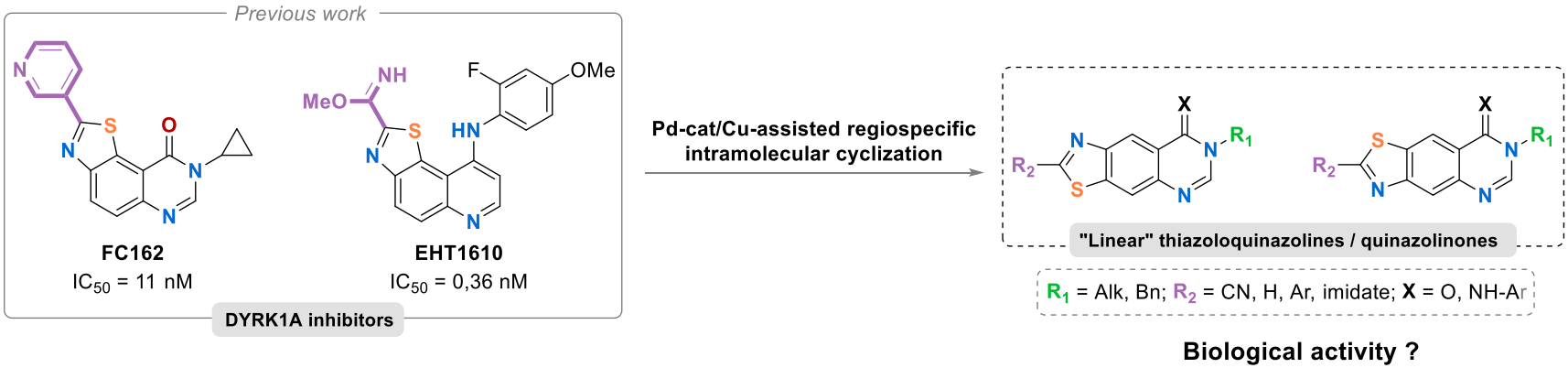
Synthesis and biological evaluation of linear thiazolo[4,5-*g*] and [5,4-*g*] quinazolines, analogues of V-shaped DYRK1A inhibitors EHT1610 and FC162



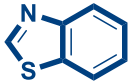
Abstract

The synthesis of new heterocyclic structures is a crucial issue in medicinal chemistry, which is constantly seeking for new active molecules. In the past 20 years, our research group has focused on the synthesis of DYRK1A inhibitors containing a thiazole ring fusionned with a quinazolin-4-one, a heterocyclic system present in many natural or synthetic molecules of biological interest. Two highly affine compounds, EHT1610 and FC162 were then identified and particularly studied. Docking studies highlighted the role of the V-shape of our compounds in their ability to inhibit DYRK1A. Recently, we described a synthetic method for access to 2-cyanobenzothiazoles from N-Arylcyanothioformamides via Pd-Catalyzed/Cu-Assisted C-H Functionalization/Intramolecular C-S bond formation. This regiospecific cyclization incited us to develop novel synthetic routes to obtain regioisomers of EHT1610 and FC162, our reference compounds. After an optimization step, this methodology allowed the synthesis of linear thiazolo[4,5-*g*] and [5,4-*g*] quinazoline regioisomers and chemical analogs of the two V-shaped leads. Preliminary results of kinase inhibition and cytotoxic evaluation of the target compounds are presented in this communication.

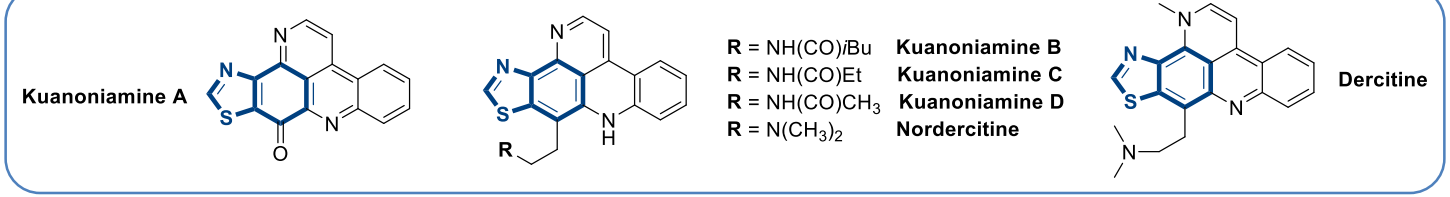
Keywords: Kinase inhibitors, Cytotoxicity, Heterocycles.

Introduction

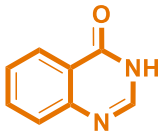
Benzothiazole motif



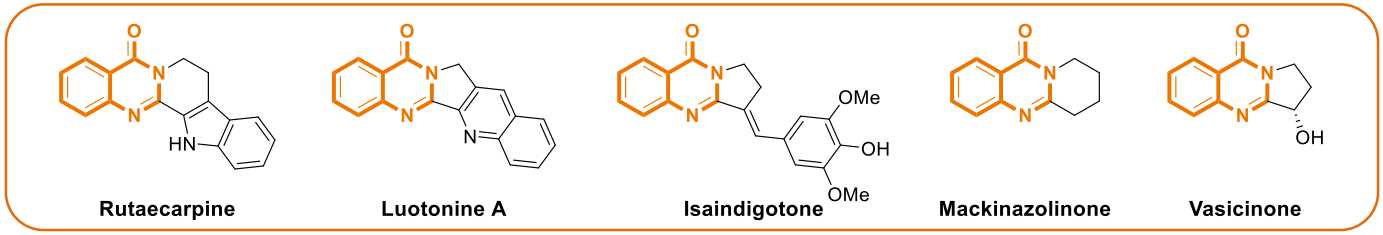
Present in natural compounds of marine origin



Quinazolinone skeleton



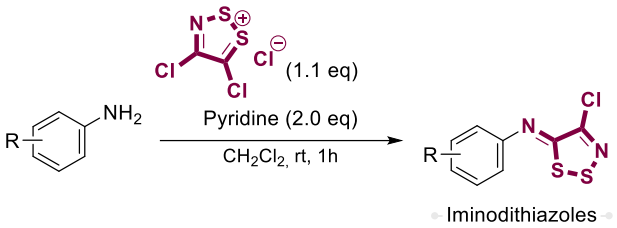
Widely studied class for their numerous biological activities (Chinese herbal medicine)



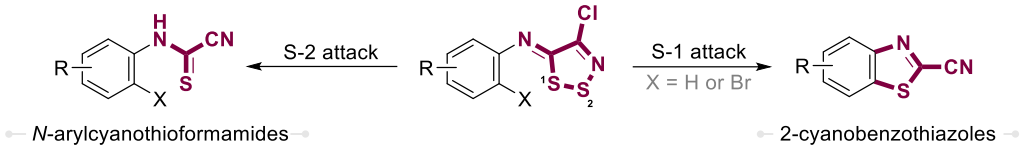
Our work : create **original** & **potentially bioactive** compounds combining these **two structures**

(a) Keri, R.S.; Patil, M.R.; Patil, S.A.; Budagumpi, S. *Eur. J. Med. Chem*. **2015**, *89*, 207–251. (b) Agarwal, S.; Gandhi, D.; Kalal, P. *Lett. Org. Chem.* **2017**, *14*, 729–742. (c) Venables, D.A.; Concepcion, G.P.; Matsumoto, S.S.; Barrows, L.R.; Ireland, C.M. *J. Nat. Prod*. **1997**, *60*, 408–410. (d) Gunasekera, S.P.; McCarthy, P.J.; Longley, R.E.; Pomponi, S.A.; Wright, A.E.; Lobkovsky, E.; Clardy, J. *J. Nat. Prod.* **1999**, *62*, 173–175.

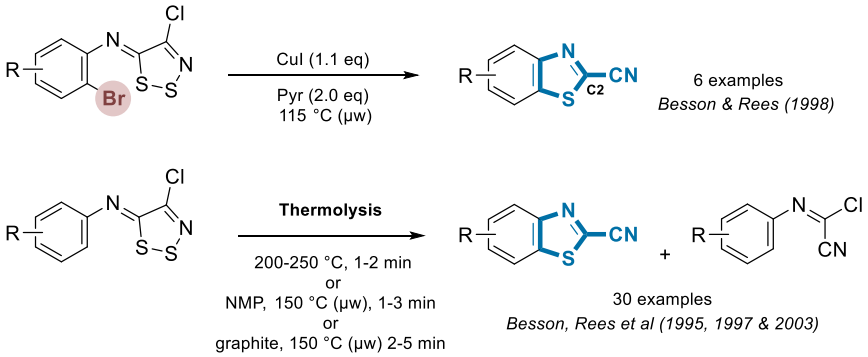
Introduction Use of a versatile reagent : Appel Salt



The iminodithiazole function obtained by the condensation of the Appel Salt on an aromatic amine presents a broad reactivity on the 2 sulfur atoms.



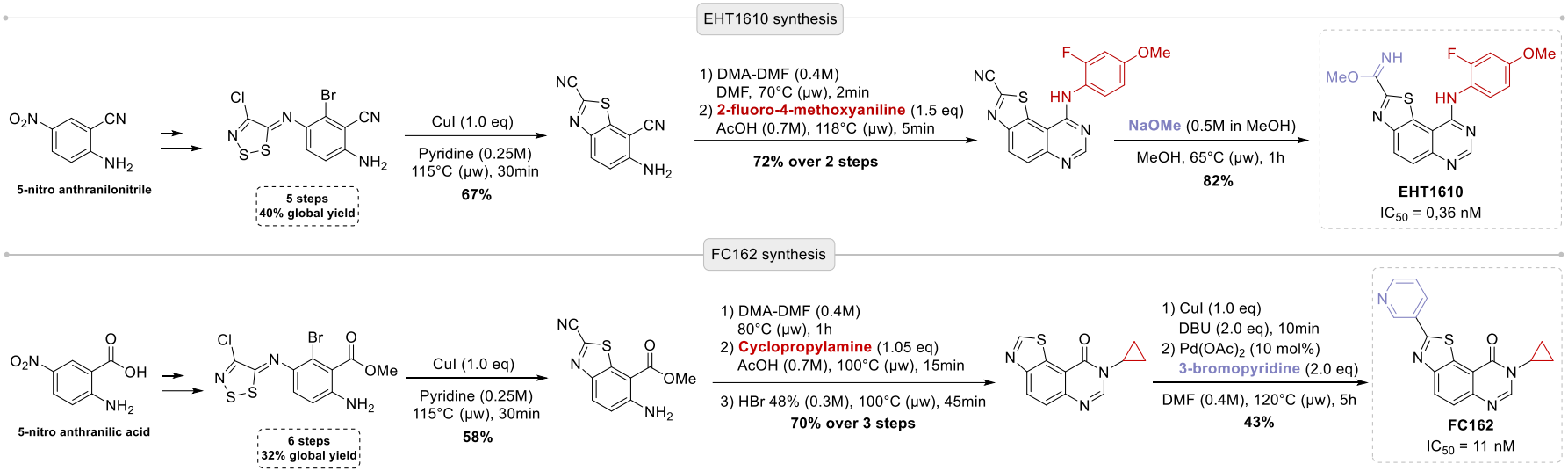
Historically, the S1 reactivity has been the most exploited to synthetize 2-cyanobenzothiazoles, following 2 different pathways, each presenting some drawbacks :

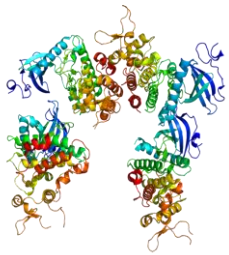


(a) Appel, R.; Janssen, H.; Knoch, F. *Chem. Ber.* **1985**, *118*. (b) Rees, C.W. *J. Het. Chem.* **1992**, *29*, 639–651; (c) Besson, T.; Rees, C.W. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 1659–1663. (d) English, R.F.; Ratikin, O.A.; Rees, C.W.; Vlasova, O. G. *J. Chem. Soc., Perkin Trans. 1*, **1997**, 201–206. (e) Bénéteau, V.; Besson, T.; Rees, C.W. *Synth. Commun.* **1997**, *27*, 2275–2280. (f) Besson, T.; Dozias, M.J.; Guillard, J.; Rees, C.W. *J. Chem.Soc., Perkin Trans. 1*, **1998**, 3925–3927. (g) Frère, S.; Thiéry, V.; Besson, T. *Synth. Comm.*, **2003**, *33*, 3795–3804.

Introduction

In our team, the copper-mediated cyclisation has been employed for the synthesis of two DYRK1A inhibitors, having a good affinity with the targeted enzymes at the nano/subnanomolar range.





DYRK1A

Dual-specificity tyrosine phosphorylation-regulated kinase

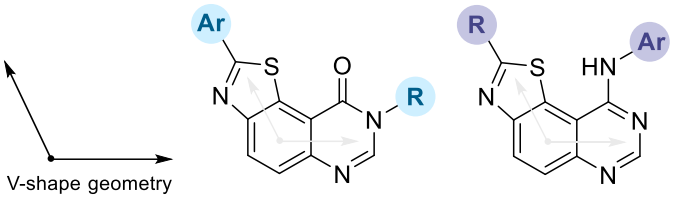
- It catalyzes its autophosphorylation on serine/threonine and tyrosine residues
- It may play a significant role in a signaling pathway regulating cell proliferation & may be involved in brain development

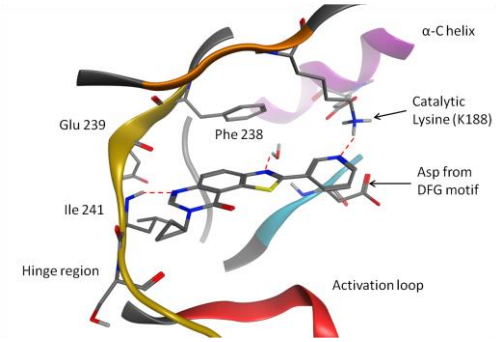
It's overexpressed in neurodenegerative diseases (Alzheimer, Down syndrome) → Target of choice

(a) Foucourt, A.; Hédou, D.; Dubouilh-Benard, C.; Girard, A.; Taverne, T.; Casagrande, A.-S.; Désiré, L.; Leblond, B.; Besson, T. *Molecules,* **2014**, *19*, 15411–15439. (b) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules,* **2018**, *23*(9), 2181–2199.

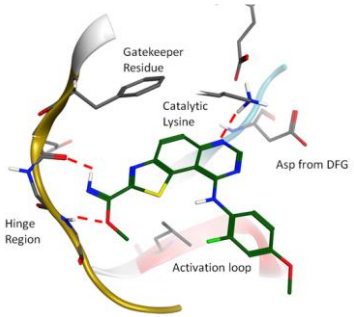
Introduction

Some RX studies of our products with DYRK showed that the V-shape geometry of our inhibitors played a significant role in the mechanism of inhibition.





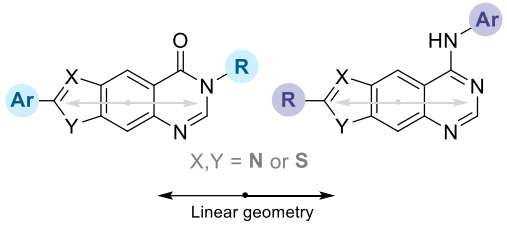
X-Ray structure of FC162-DYRK1A co-crystal (PDB-6QU2)



X-Ray structure of EHT1610-DYRK2 co-crystal (PDB-5LXD)

Knapp et al.

If we succeed in the development of a new reactional pathway for the linear analogues of FC162/EHT1610…

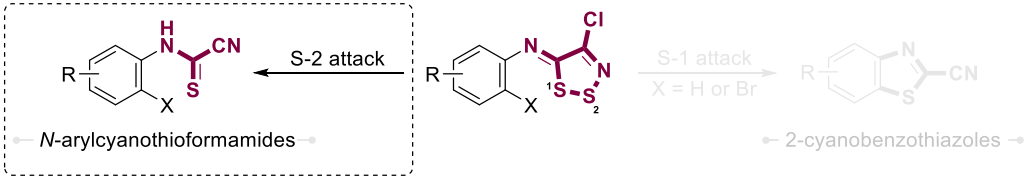


Linear analogues of FC162/EHT1610

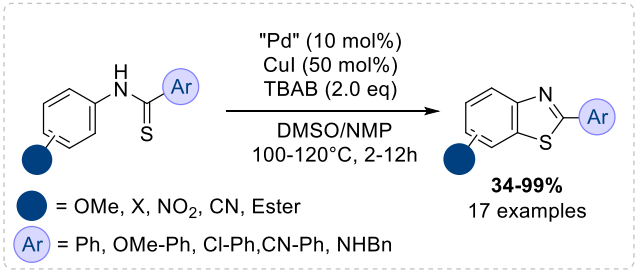
What would be the impact of 'linear' geometry on kinase inhibition?

Introduction

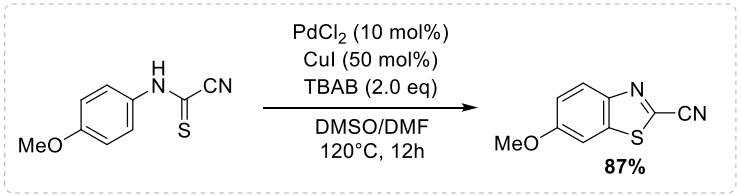
For this, we wanted to exploit the S2 reactivity of iminodithiazoles to synthesize *N*-arylcyanothioformamides.



In literature, there are only few examples of cyclisation of this function or similar ones
The two examples below will be the starting point of our optimization :



Doi, **2008**



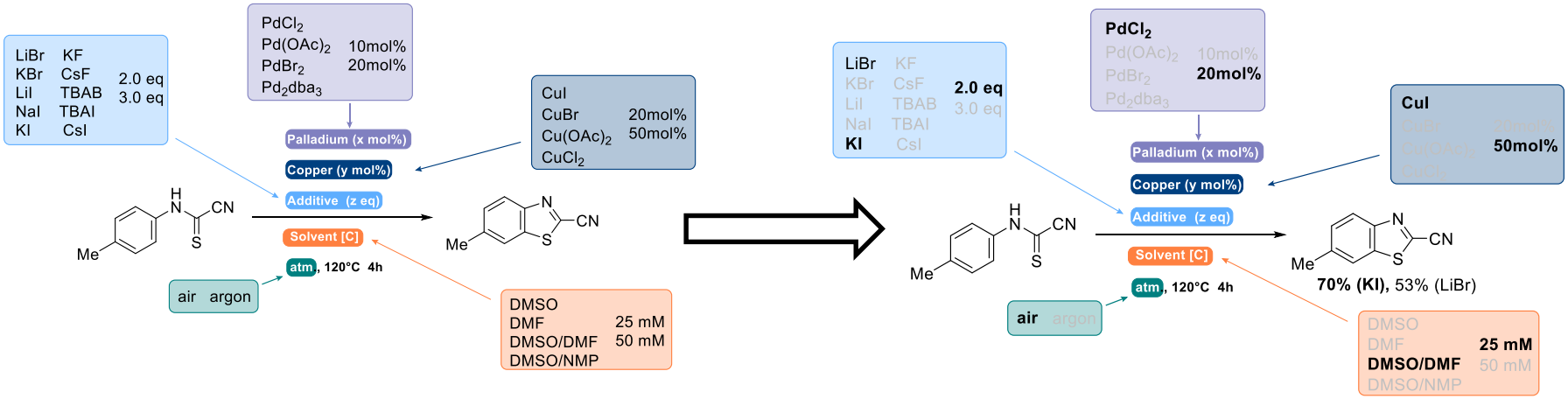
Prescher, **2012 (Only one example)**

Results and discussion

Our optimisation begun first by the synthesis of a large array of *N*-arylcyanothioformamides, while using Koutentis method with an excess of DBU.

Koutentis, **2009**

While using Doi's conditions as a starting point, we screened all the different parameters of this intramolecular cyclisation in order to identify the optimized conditions

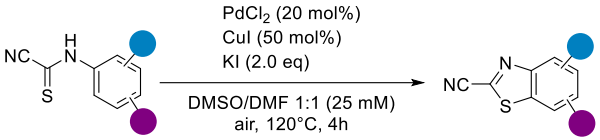


$PdCl_2$ (20 mol%), CuI (50 mol%), KI (2.0 eq) in a 1:1 mixture of DMSO/DMF (25 mM) under air atmosphere were identified as the best conditions here. However, we will have to keep in mind that LiBr is also a great additive for this reaction, and will be very usefull for the continuation of this work.

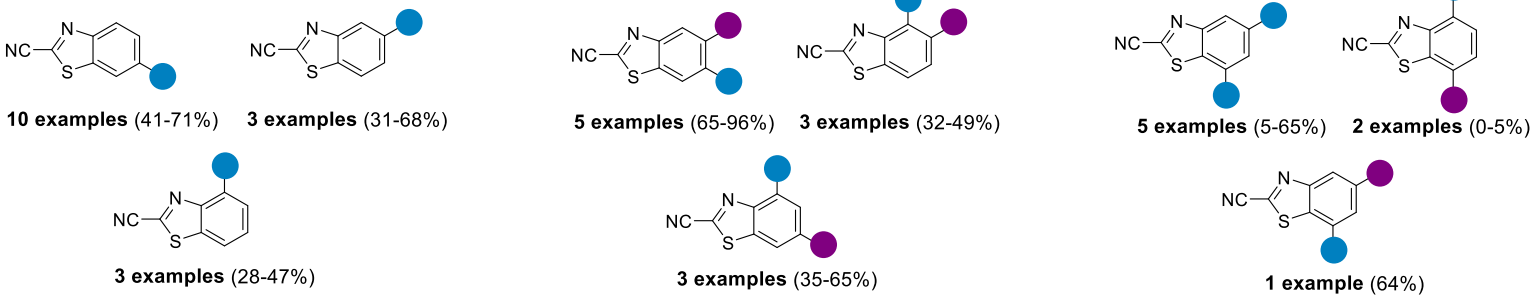
(a) Michaelidou, S. S.; Koutentis, P. A. *Synthesis*, **2009**, *24*, 4167–4174. (b) Broudic, N.; Pacheco-Benichou, A.; Fruit, C.; Besson, T. *Molecules*, **2022**, *27*, 8426–8444.

Results and discussion

Our optimized conditions were applied to a large array of *N*-arylcyanothioformamides, to obtain more than 30 novel 2-cyanobenzothiazoles, in average good yields.



Scope

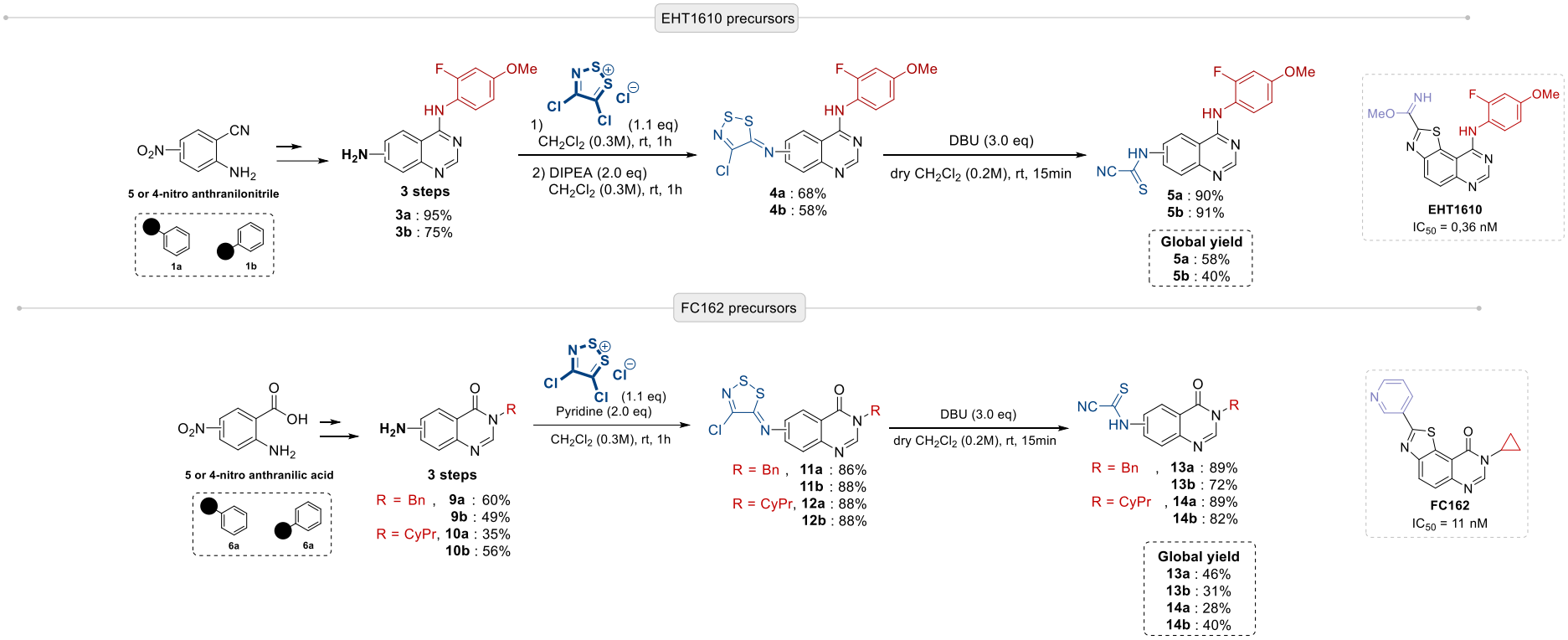


, = Me, OMe, X (Cl, Br, F), NO_2, CF_3, CN, $-OCH_2O-$, $-O(CH_2)_2O-$,ester

For unsymmetrical *N*-arylcyanothioformamides, we succeeded to cyclize only at the least hindered position, making this method regioselective.

Results and discussion

Then, we had to synthetize the *N*-arylcyanothioformamides precursors of our linear analogues of FC162 and EHT1610. For each, the first 3 steps were already optimized. After 2 new steps of Appel Salt condensation and DBU treatment, we were allowed to obtain 6 new precursors, in good yield.

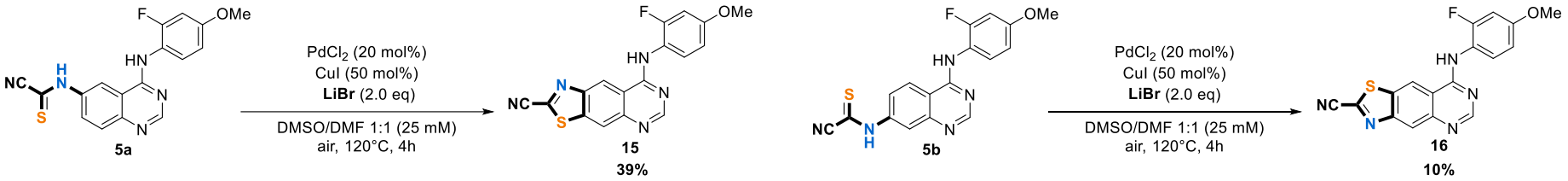


a) Foucourt, A.; Hédou, D.; Dubouilh-Benard, C.; Girard, A.; Taverne, T.; Casagrande, A.-S.; Désiré, L.; Leblond, B.; Besson, T. *Molecules,* **2014**, *19*, 15411–15439. b) Couly, F.; Harari, M.; Dubouilh-Benard, C.; Bailly, L.; Petit, E.; Diharce, J.; Bonnet, P.; Meijer, L.; Fruit, C.; Besson, T. *Molecules,* **2018**, *23*(9), 2181–2199.

Results and discussion

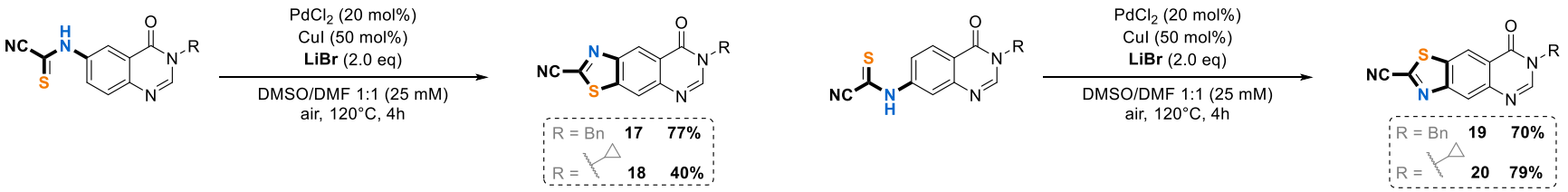
Our 6 precursors were engaged in our cyclisation method. After some trials (details are not given here), we observed that LiBr is the best additive for those more complex substrates.

EHT1610 precursors



The 2 desired products were obtained in low yields but regioselectively, confirming results obtained during the reaction optimisation.

FC162 precursors

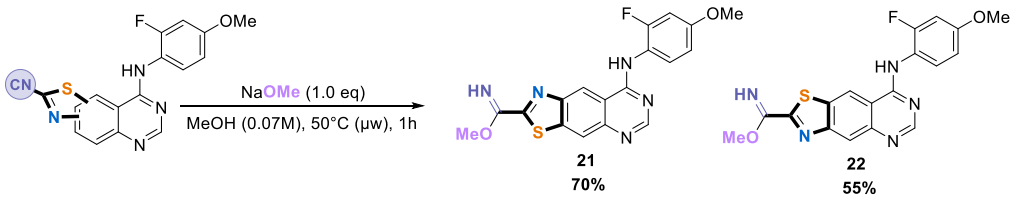


4 new quinazolinones were obtained regioselectively in good yields, allowing to obtain 6 new precursors of linear analogues of FC162 and EHT1610.

Results and discussion

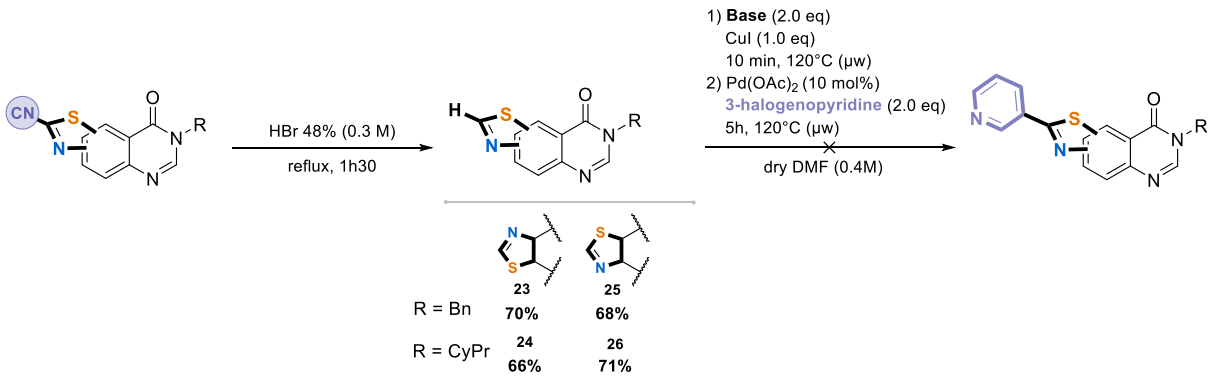
In order to obtain exact regioisomers of FC162 and EHT1610, we had to modulate the carbonitrile part.
For EHT1610 analogues, our cyano precursors were reacted with sodium methoxide in methanol to obtain corresponding imidates.

EHT1610 analogues



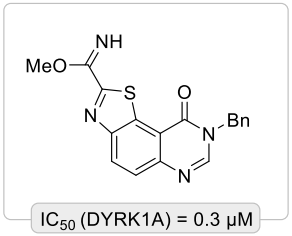
For FC162 regioisomers, our precursors were first decyanated in acidic aqueous medium, and then they were engaged in C-H arylation conditions developped by our group. Unfortunately, we were never able to isolate arylated products.

FC162 analogues



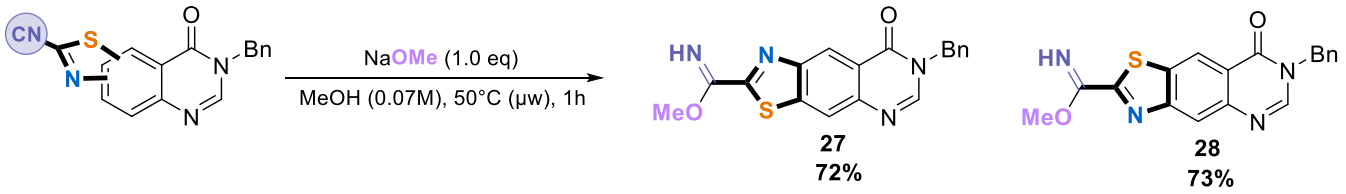
Results and discussion

During her PhD thesis in our laboratory, Dr. Florence Couly identified the following imidate compound as a good inhibitor of DYRK1A.



F. Couly PhD Thesis

In order to establish comparisons for biological tests, we synthesized linear analogues of this compound, using our cyanoquinazolinone precursors under the conditions already described for the EHT1610 analogues, 2 novel imidate derivatives were obtained in good yields.



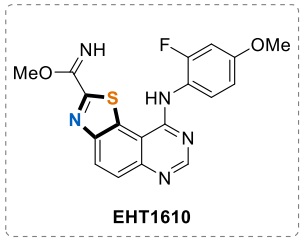
Results and discussion

The DYRK1A inhibition evaluation was performed, and results are described below.

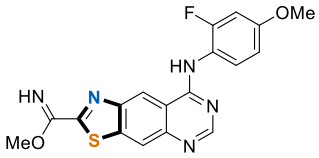
Compared with their angular analogues, linear compounds are inactive on DYRK1A inhibition. These results were also observed on the panel of 8 kinases tested. It confirms the importance of the V-shape as it was observed during previous co-crystallisation studies.

Thomas Robert
Dr. Stéphane Bach

CNRS UPMC Station Biologique Roscoff



EHT1610



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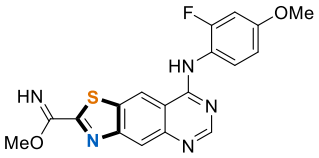
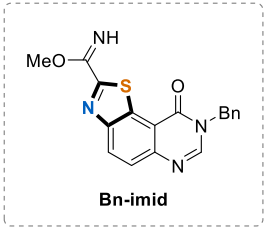
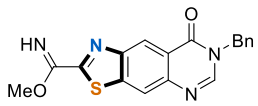


22

	Concentration	EHT1610	21	22
Residual activity of DYRK1A (%)	10 µm	15	27	≥100
	1 µm	16	72	95



Bn-imid



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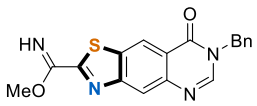

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	Concentration	Bn-Imid	27	28
Residual activity of DYRK1A (%)	10 µm	21	≥100	≥100
	1 µm	65	≥100	≥100

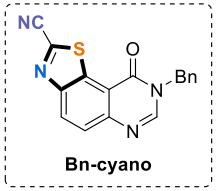
Kinases panel

CDK9
HASPIN
PIM1
GSK3β
CK1e
JAK3
CLK1

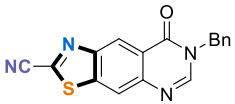
Hélène Solhi
Dr. Rémi Le Guével

Results and discussion

Wishing to add value to those new linear compounds, we wanted to evaluate their cytotoxicity, thanks to a collaboration with Dr. Rémi Le Guével at Rennes 1 University. More than 25 compounds were tested on 7 cancerous cell lines and 1 healthy cell line (fibroblasts). The 3 compounds described below are the most active. They are substituted by a carbonitrile or an imidate function at the C2 position.



Bn-cyano



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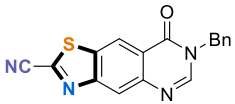

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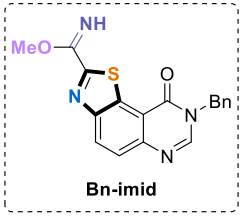
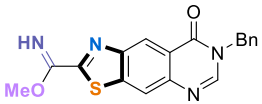


Bn-imid



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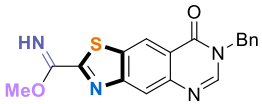
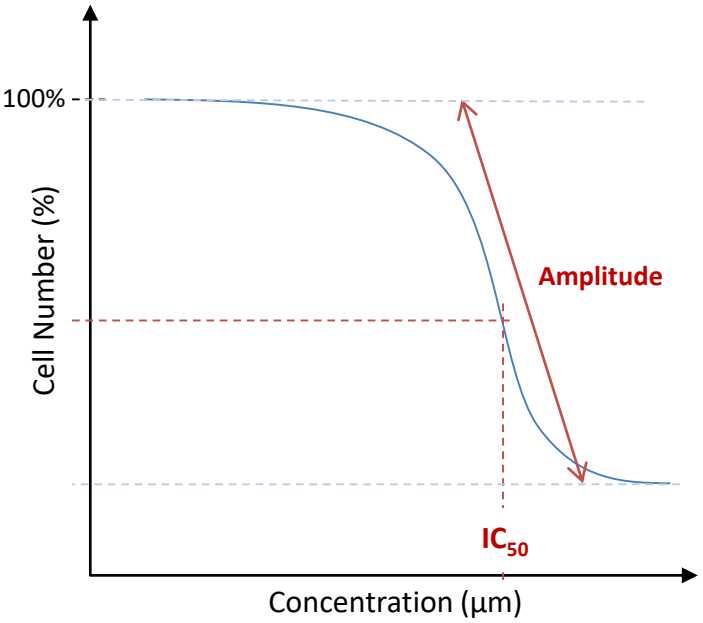


28

Cyano series

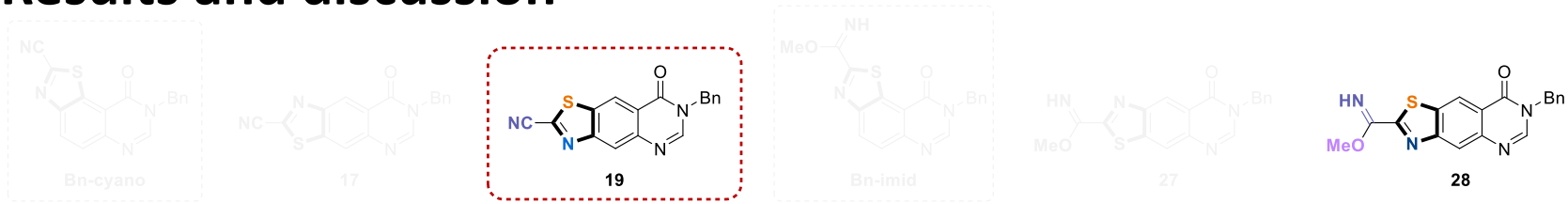
Imidate series

	Bn-cyano	17	19	Bn-imid	27	28
Cell lines	IC_{50} (µM)/Amplitude					
HuH7 (liver)	13/78	62/73	3/94	15/58	27/63	1/54
CaCo-2 (colon)	10/83	10/54	1/86	12/71	17/59	2/36
MDA-MB-231 (breast)	10/87	40/74	3/91	21/56	20/51	3/71
MDA-MB-462 (breast)	10/87	40/74	1/99	21/56	20/51	3/71
HCT116 20X (colon)	7/99	10/80	2/92	18/99	20/91	1/95
PC3 (prostate)	11/95	9/65	2/98	20/75	20/67	2/72
MCF7 (breast)	13/95	8/67	2/98	17/80	17/67	1/73
Fibroblasts	8/40	16/33	1/36	5/28	7/33	4/47



Hélène Solhi
Dr. Rémi Le Guével

Results and discussion



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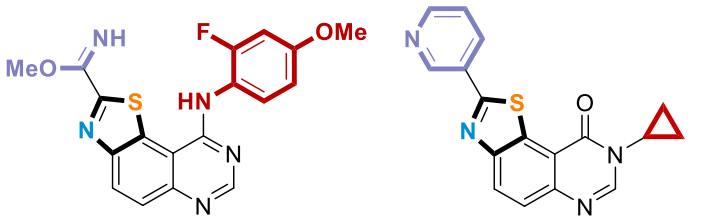
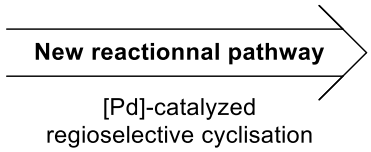
Compound 19 exhibits the best cytotoxicity profile, with IC_{50} at the micromolar range. Comparison with the imidate analogue, shows a decrease of the amplitude value, indicating a decrease of the cytotoxicity. This observation confirms the importance of the carbonitrile part on the C2 position, and allows us to identify linear thiazoloquinazolinone 19 as a structure of interest.

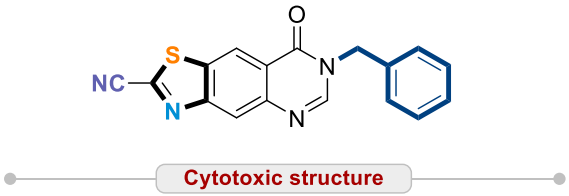
Conclusions During this work were performed :

During this work were performed :

- ☑ Development of a regioselective cyclisation of *N*-arylcyanothioformamides (Appel Salt Chemistry)
- ☑ New reactionnal pathway for the synthesis of linear analogues of EHT1610/FC162
- ☑ Biological evaluation → **Identification of a structure of interest**







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