

Reactivity Under Microwave Irradiation of 2-amino 4H-chromene-3-carbonitrile as tool for the construction of potential bioactive derivatives

Ali Bouattour, Mehdi Fakhfakh, Houcine Ammar, Ludovic Paquin, Jean-Pierre Bazureau, Rémy Le Guével, Anne Corlu, Souhir Abid, Sandrine Ruchaud, Stéphane Bach

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Reactivity under microwave irradiation of 2-amino 4*H*-chromene-3-carbonitrile as tool for the construction of potential bioactive derivatives.

Jean-Pierre Bazureau [1,5]*, Ali Bouattour [2], Mehdi Fakhfakh [2], Souhir Abid [2], Houcine Ammar [2], Ludovic Paquin [1,5], Rémy Le Guével [3], Anne Corlu [3], Sandrine Ruchaud [4] and Stéphane Bach [4]

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2 Laboratoire de Chimie Appliquée: Hétérocycles Corps Gras & Polymères, Université de Sfax, Route Soukra BP 1171, 3000 Sfax (Tunisie)

3 ImPACcell platform, SFR Biosit, Université de Rennes 1, Bât. 8, Campus de Villejean, 2 Av. du Prof. Léon Bernard, CS 34317, 35043 Rennes Cedex (France)

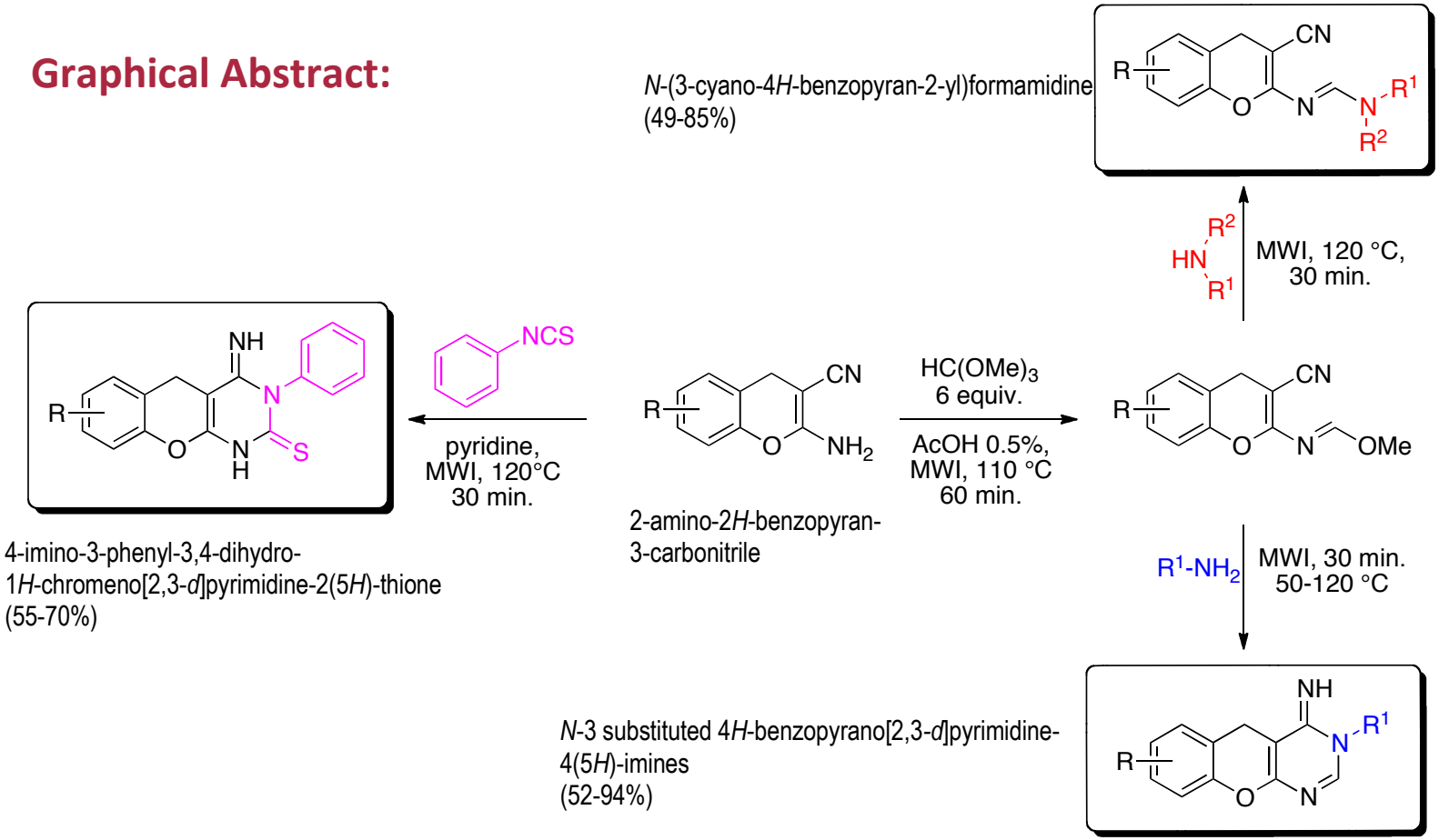
4 Station Biologique de Roscoff, USR 3151, CNRS-UPMC, *Kissf* platform, Place Georges Tessier, BP 74, 29682 Roscoff (France)

5 Université de Rennes 1, S2 Wave platform, ScanMat UMS 2001 CNRS, Bât. 10A, Campus de Beaulieu, 263 Avenue du Gén. Leclerc, CS 74205, 35042 Rennes Cedex (France)

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Reactivity under microwave irradiation of 2-amino 4*H*-chromene-3-carbonitrile as tool for the construction of potential bioactive derivatives.

Graphical Abstract:

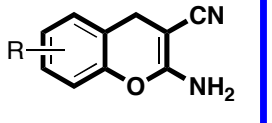


Abstract: The interest of 4*H*-chromenes (or 4*H*-benzopyranes) and their derivatives are components of many naturally occurring products, which have also been submitted to structural modifications to increase molecular diversity, for potential medicinal properties. In this context and starting from the 2-amino-2*H*-benzopyran-3-carbonitrile platform, it was possible to built easily (20 min.) a new class of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thiones (55-70%) under microwave at 120°C in pyridine medium. Treatment of the amino 4*H*-chromene platform with orthoester gave the corresponding methanimidate intermediate, which is converted into formamidine derivatives (63-85%) from various cyclic secondary amines or, into *N*-3 substituted 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imines (49-94%) under microwave irradiation (50-120─C, 30 min.). The biological properties of all products were explored by *in vitro* cancer assays against a panel of seven tumor cell lines (Huh 7D12, Caco2, MDA-MB231, HCT116, PC3, NCI-H727, HaCat, fibroblasts which are representative of different cancers: leukemia, melanona, and cancers of liver, colon, breast, prostate, lung, and kidney) and also, *in vitro* Serine/Threonine protein kinase inhibition assays (*Hs*CDK5-p25, GSK3α/β, CLK1, *Hs*Haspin, *Hs*PIM1, *Hs*Aurora B). Some of these 2-imino- or 2-amino-2*H*-benzopyran-3-carbonitriles are active against tumor cell lines (Huh7, Caco 2, HCT 116) or protein kinases (CLK1).

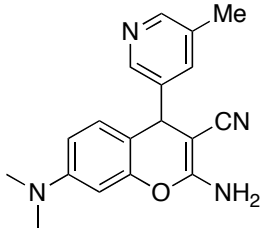
Keywords: microwave / chromeno[2,3-*d*]pyrimidine / benzopyran-2-yl methanimidate/ formamidine.

The 2-amino-4H-benzopyran scaffold.....an interesting structure open to bioactive derivatives !

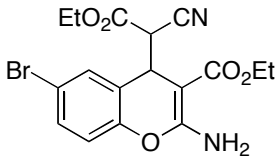
Introduction



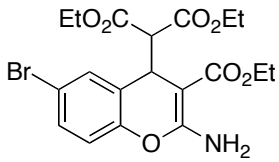
2-amino-2*H*-benzopyran-3-carbonitrile



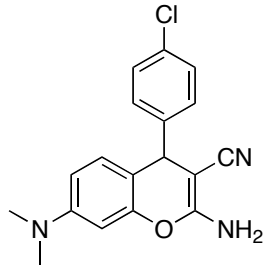
MX58151
anticancer agent
J. Med. Chem. **2008**, *51*, 417



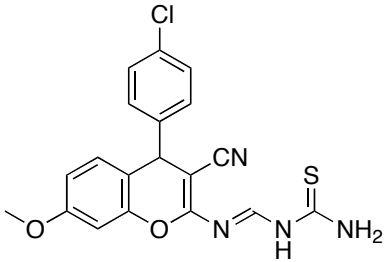
HA 14-1
Bcl-2 inhibitor
Tetrahedron **2009**, *65*, 10149
Leukemia Res. **2007**, *31*, 859



SV30
Bcl-2 inhibitor
J. Control. Rel. **2011**, *151*, 74

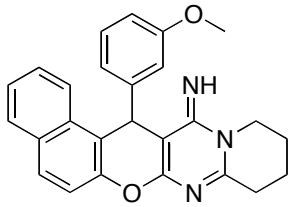


compound **3a**
anticancer activity
MCF cell line IC_{50} 19.7 μM
Eur. J.. Med. Chem. **2011**, *46*, 765



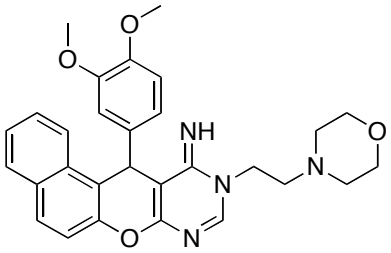
(R, S)-N'-(4-(4-chlorophenyl)-3-cyano-7-methoxy-4*H*-chromen-2-yl)-*N*-carbamoylformamimidamide

MCF7 cell line IC_{50} 8 nM
Der Pharm. Chem. **2012**, *4*, 1653

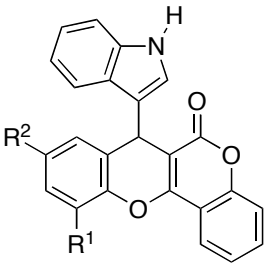


15-(3-methoxyphenyl)-9,11,12,15-tetrahydro-10*H*-14*H*-benzo[5,6]chromeno[2,3-d]pyrido[1,2-*a*]pyrimidine-14-imine

$A\beta_{1\text{-}42}$ aggregation inhibitor
*h*AchE IC_{50} 58 nM & *h*BuChe IC_{50} 302 nM
ChemMedChem **2016**, *11*, 1318

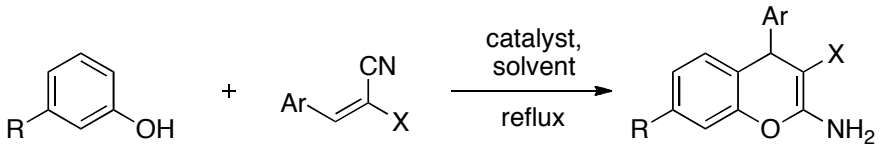


NPSR antagonist
cAMP IC_{50} 4.87 μM
ACS Chem Neurosci. **2010**, *1*, 559



7-(1*H*-indol-3-yl)-6*H*,7*H*-chromeno[4,3-*b*]chromen-6-one
R^1 = H, Br, I
R^2 = H, Br, I, NO_2, MeO
anti HIV activity
Synlett **2015,** *26*, 1969

The usual methods to build the 2-amino-4*H*-benzopyran derivatives by Michaël addition and cyclisation



R = Et_2N, OH, OMe

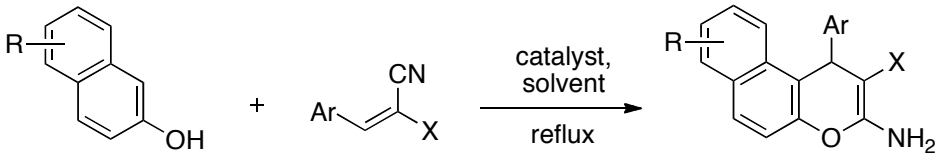
X = CN, CO_2Et

with always substituent in position C-4 !

Catalyst & solvent used:

- piperidine, EtOH: *Eur. J. Med. Chem.* **2011**, *46*, 765
- Fe_2O_3, magnetic nanoparticules, H_2O: *Phosphorus, Sulf. and Silicon* **2014**, *189*, 1
- K_2CO_3, EtOH: *J. Chem. Pharm. Res.* **2009**, *1*, 213
- $InCl_3$, H_2O/EtOH: *Tetrahedron Lett.* **2007**, *48*, 6785
-))) irradiation, Fe_3O_4-chitosan MNPs, H_2O: *Ultrason. Sonoch*em. **2015**, *22*, 341

And extension to 2-amino-4*H*-benzo[*h*]chromene derivatives



R = OH, OMe

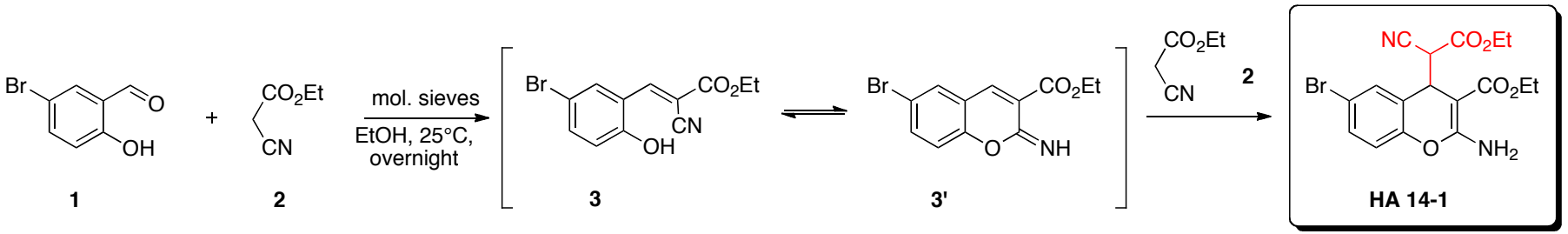
X = CN, CO_2Et

Catalyst & solvent used:

- PEG-400: *Green Chem. Lett. Rev.* **2010**, *3*, 83
- Piperidine, EtOH: *Pharmaceuticals* **2012**, *5*, 745 & *Med.Chem.Res.* **2012**, *26*, 691

Other methods to build the 2-amino-4*H*-benzopyran derivatives

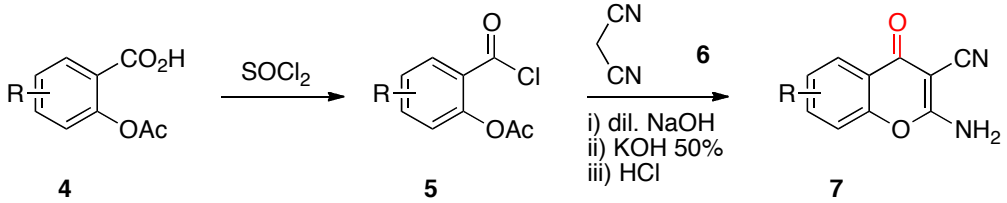
Tetrahedron Lett. **2008**, *49*, 3276-78



*via iminocoumarine **3'** .....applied to the synthesis of **HA 14-1***

Or using *aspirin* derivatives as starting material

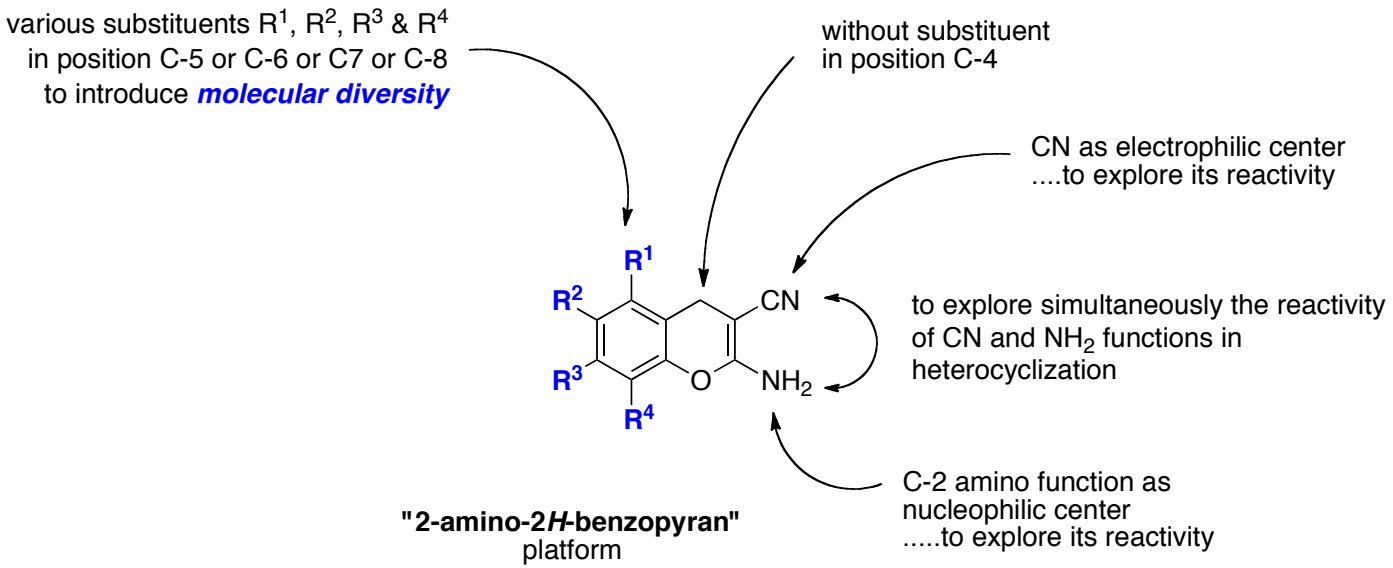
J. Sulfur Chem. **2011**, *32*, 451-62



with C=O in position C-4

Our major interests on 2-amino-4*H*-benzopyran platform:

1. To build the 2-amino-4*H*-benzopyran platform *via* iminocoumarin chemistry using simple methods of organic chemistry,
2. To prepare original compounds after heterocyclization between CN and NH_2 functions,
3. To explore introduction of amidine function using NH_2 in C-2 position….to obtain original formamidine derivatives
4. Using microwave irradiation to reduce reaction time, to increase chemical yields and qualities of the new products



Our interest on 2-amino-4*H*-benzopyran scaffold and their derivatives….

As potential protein kinase inhibitors (on 6 selected PKs)

CLK1: Dual specificity protein kinase is an enzyme in human encoded by the CLK1 gene

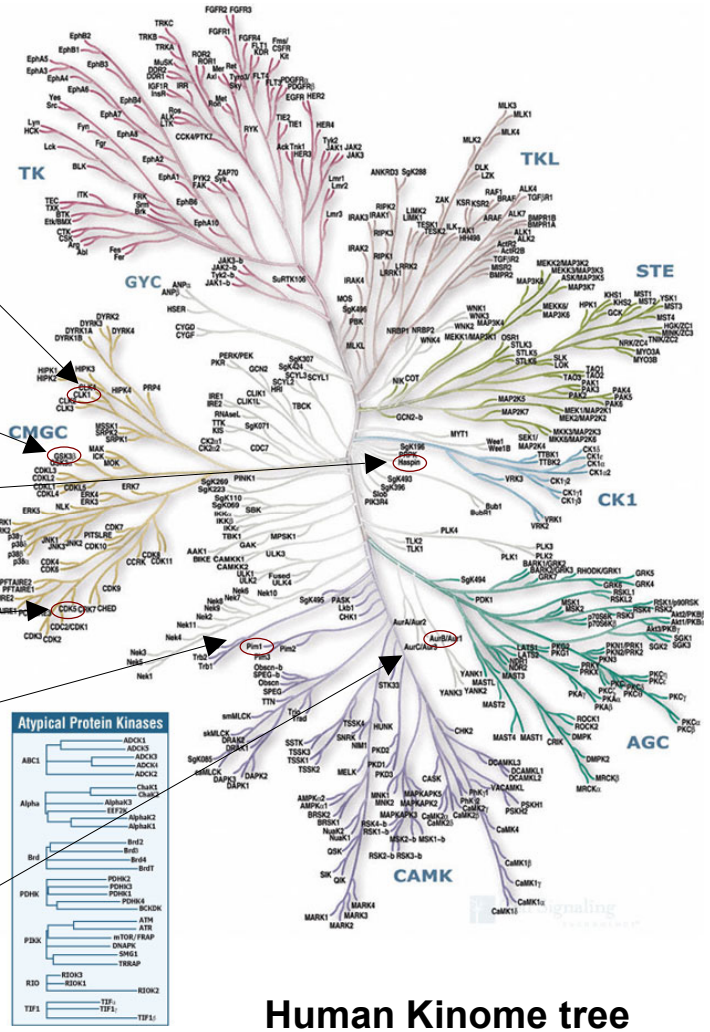
GSK3α/β: is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and theronine amino acid residues

***Hs*Haspin:** is a serine/threonine protein kinase encoded by the *GSG2* gene

***Hs*CDK5-p25:** (Cyclin-dependant kinase 5) is an enzyme encoded by the *CDK5* gene.

***Hs*Pim1:** Proto-oncogene serine/threonine protein kinase encoded by the Pim1 gene

***Hs*Aurora B:** is a protein that functions in the attachment of the mitotic spindle to the centromere



Human Kinome tree

What is a protein kinase ?

A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation). Phosphorylation usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins.

What is a protein kinase inhibitor ?

Deregulated kinase activity is a frequent cause of disease, in particular cancer, wherein kinases regulate many aspects that control cell growth, movement and death. *Drugs that inhibit specific kinases are being developed to treat several diseases, and some are currently in clinical use.*

What is human kinome tree ?

The human kinome tree represents the complete set of the 518 human protein kinases encoded in its genome and they constitute about 2% of all human genes.

3rd International Electronic Conference on Medicinal Chemistry
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Our interest on 2-amino-4*H*-benzopyran scaffold and their derivatives….

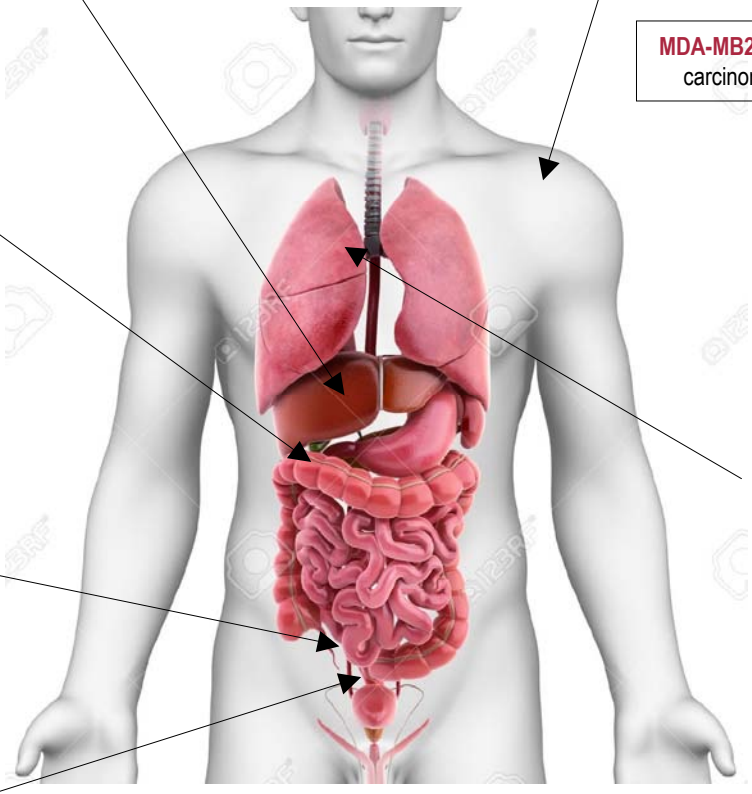
Against selected tumoral cell lines

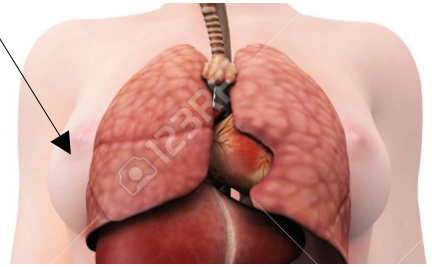
Huh7-D12 cell (differential hepato-cellular carcinoma) from human liver

HaCaT cell (aneuploid immortal keratinocyte cell) from adult human skin

MDA-MB231 cell (invasive ductal carcinoma) from woman breast

Caco2 cell (epithelial colorectal adenocarcinoma) from human large intestine





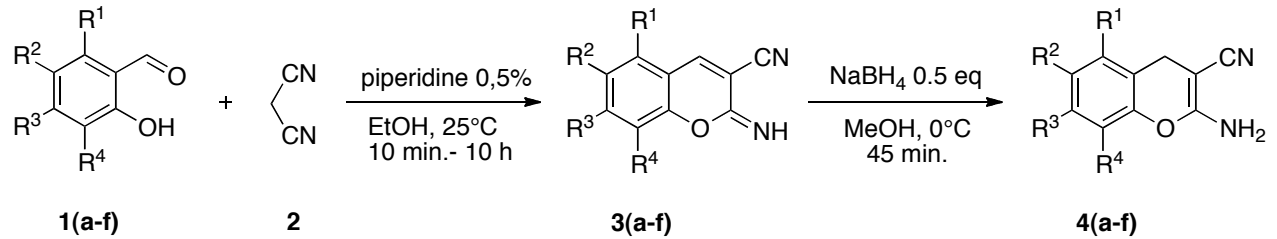
NCI-H727 cell (bronchial carcinoma) from human lung

HCT116 cell (actively proliferating colorectal carcinoma) from human colon

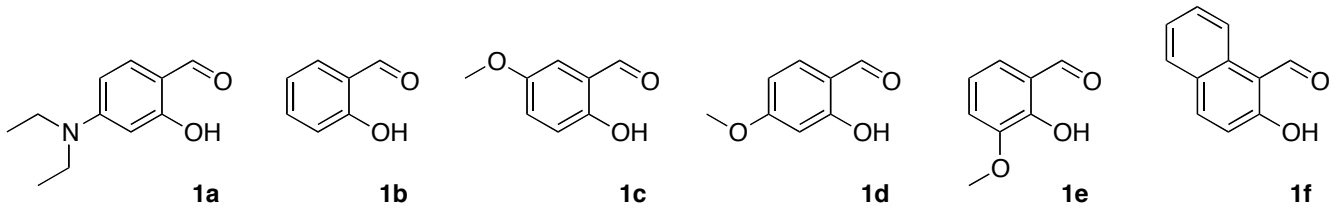
PC3 cell (prostatic carcinoma) from human prostate

Results and discussion

Part 1: Preparation of various 2-amino-4*H*-benzopyran *via* 2-imino-2*H*-benzopyran



1(a-f) **2** **3(a-f)** **4(a-f)**



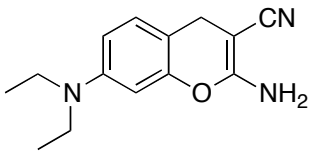
1a **1b** **1c** **1d** **1e** **1f**

Remark: in the 1st step, piperidine (0.5%) is used as catalyst

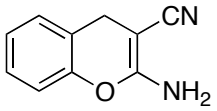
A. Bouattour et al., *Arkivoc* **2017**, *iv*, 291-302
M. Fakhfakh et al., *Dyes Pigments* **2010**, **84**, 108-113

Part 1.1: Results for the synthesis of 2-amino-4*H*-benzopyran 4(a-f)

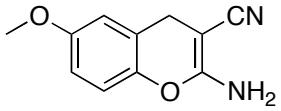
Access to 2-amino-4H-benzopyran platform is very easy !



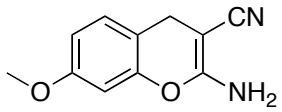
4a: 70% yield
$\delta_{H\text{-}4}$ = 3.25 ppm



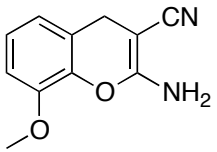
4b: 82% yield
$\delta_{H\text{-}4}$ = 3.45 ppm



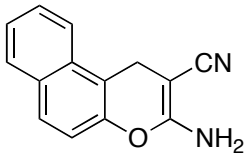
4c: 50% yield
$\delta_{H\text{-}4}$ = 3.42 ppm



4d: 90% yield
$\delta_{H\text{-}4}$ = 3.37 ppm



4e: 63% yield
$\delta_{H\text{-}4}$ = 3.43 ppm



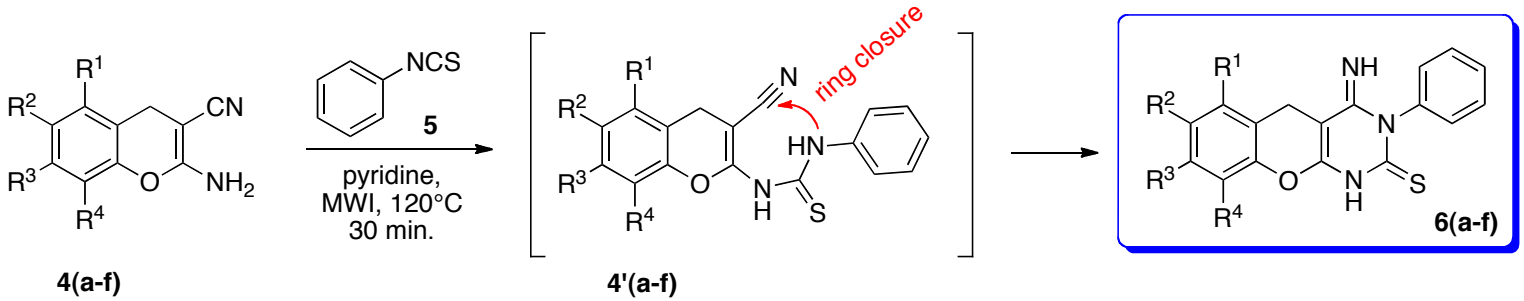
4f: 95% yield
$\delta_{H\text{-}4}$ = 3.77 ppm

Good yields for reduction with $NaBH_4$ in the 2nd step: 50-95%

Characteristic signal for ^{1}N NMR in DMSO-d_6 solution: CH_2 in H-4 position, $3.25 < \delta_{H\text{-}4} < 3.77$ ppm

A. Bouattour et al., *Arkivoc* **2017**, *iv*, 291-302

Part 1.2: Transformation of 2-amino-4*H*-benzopyran 4(a-f) into 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f)



Heterocyclization *(addition + ring closure)* ***works well only under microwave irradiation*** *in basic media (pyridine)*
Short reaction time (30 min.) + moderate temperature (120°C)
= good yields and overall yields for ***6(a-f)***



No reaction occurs in oil bath with another reaction media

Thanks to microwave !





Reaction vials:
10 & 30 mL
with snap caps

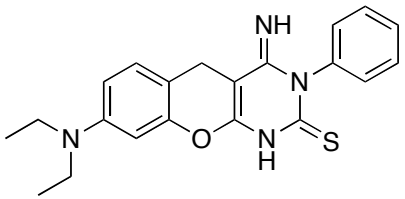
Microwave reactor

Monowave 300
(850 Watt)

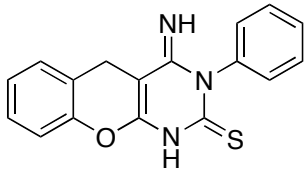
Anton Paar

A. Bouattour et al., *Arkivoc* **2017**, *iv*, 291-302

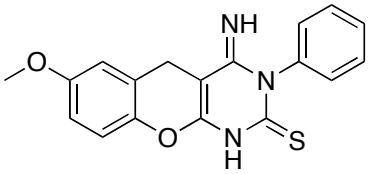
Part 1.3: Results for the preparation of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f)



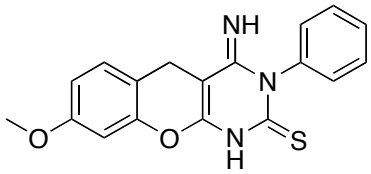
6a: 60% yield
25% overall yield
$\delta_{H\text{-}4}$ = 3.56 ppm



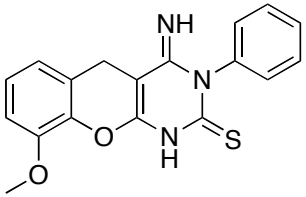
6b: 67% yield
44% overall yield
$\delta_{H\text{-}4}$ = 3.73 ppm



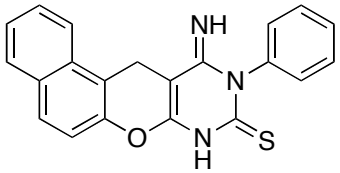
6c: 70% yield
21% overall yield
$\delta_{H\text{-}4}$ = 3.72 ppm



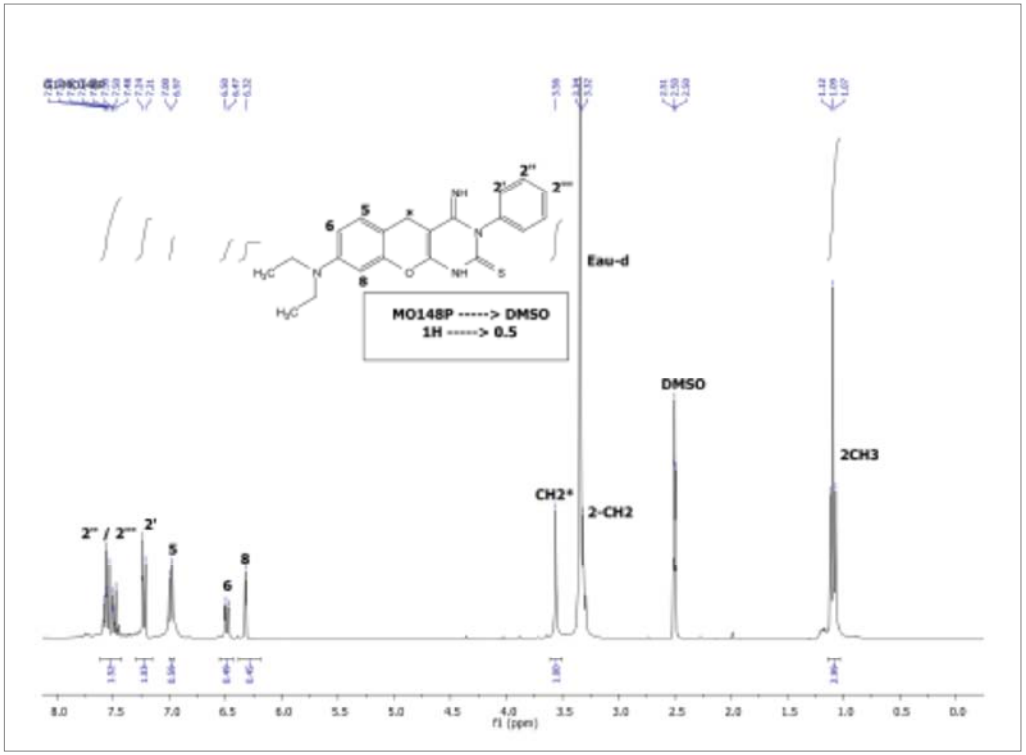
6d: 65% yield
41% overall yield
$\delta_{H\text{-}4}$ = 3.64 ppm



6e: 65% yield
41% overall yield
$\delta_{H\text{-}4}$ = 3.72 ppm



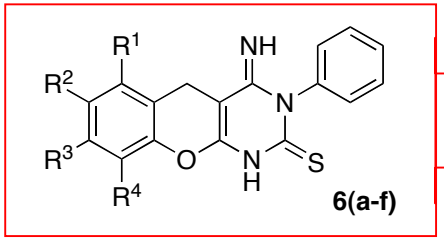
6f: 55% yield
50% overall yield
$\delta_{H\text{-}4}$ = 3.94 ppm



*^{1}H NMR spectrum of 8-diethylamino-4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (**6a**)*

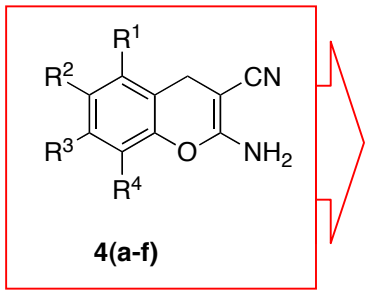
A. Bouattour et al., *Arkivoc* **2017**, *iv*, 291-302

Part 1.4: Effects of compounds 3(a-f), 4(a-f) and 6(a-f) on the catalytic activity of protein kinases and antiproliferative activity on tumor cell lines

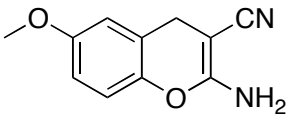


6(a-f)

None of compounds **6(a-f)** presented a significant toxicity (IC_{50} > 25 μM) on tumoral cell lines (Huh7 D12, Caco2, MDA-MB231, HCT 116, PC3, NCI-H727 and HaCaT) and no Inhibitory activity on protein kinases (*Hs*CDK5-p25, GSK3α/β, CLK1, *Hs*Haspin, *Hs*Pim1 and *Hs*Aurora B).

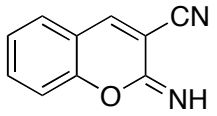


4(a-f)

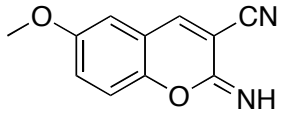


4c
CLK1: IC_{50} 0.9 μM

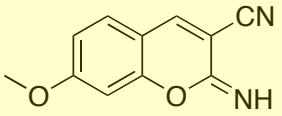
*Only the 2-amino-4H-benzopyran-3-carbonitrile **4c** inhibited selectively the protein kinase CLK1*



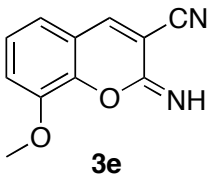
3b
Huh7: IC_{50} 2 μM
Caco2: IC_{50} 6 μM



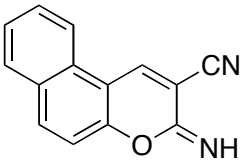
3c
Caco2: IC_{50} 6 μM
CLK1: IC_{50} 1.5 μM



3d
CLK1: IC_{50} 0.25 μM



3e
CLK1: IC_{50} 0.5 μM

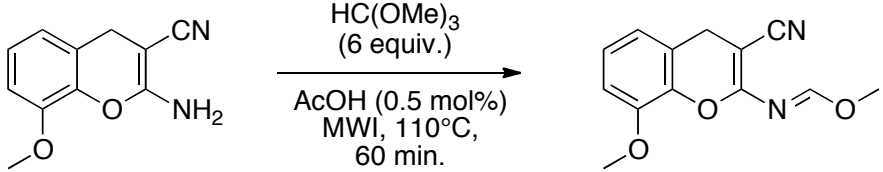


3f
Caco2: IC_{50} 9 μM
HCT 116: IC_{50} 9 μM

Selective and submicromolar inhibitor of protein kinase CLK1

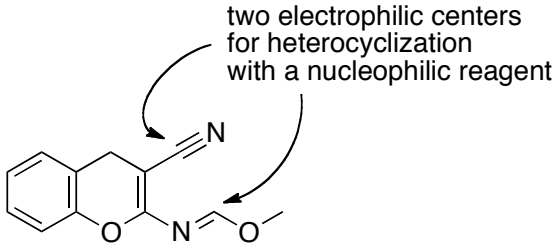
A. Bouattour et al., *Arkivoc* **2017**, *iv*, 291-302

Part 2: Interest of microwave irradiation for transformation of 2-amino-4*H*-benzopyran 4e into γ-bilectrophile methyl methanimidate 7



4e

7: 51% yield



7 as γ-bielectrophile

✓ In oil bath after 24 hrs with various acidic catalysts at 110°C: ~ 5-10% for **7**



Thanks to microwave !

✓ Under **microwave irradiation** in Monowave 300 Anton-Paar apparatus:



- Only 0.5 mol% of AcOH as catalyst
- But 6 equiv. of $HC(OMe)_3$.....as a cheap reagent !
- After 60 min. of irradiation at 110°C:

  7 is completely insoluble……separation by simple filtration

  And it's possible to scale the synthesis in multi-grams





Reaction vials:
10 & 30 mL
with snap caps

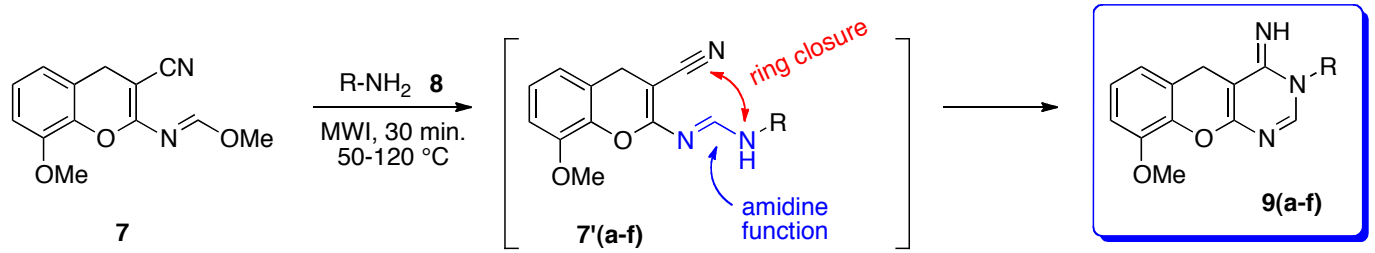
Microwave reactor

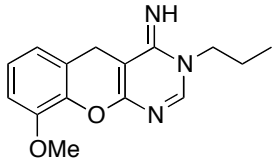
Monowave 300
(850 Watt)

Anton Paar

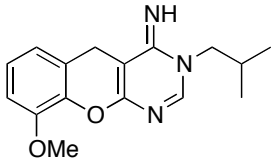
A. Bouattour et al., *Synthesis* **2017**, *49*, 3768-3774

Part 2.1: Microwave synthesis of 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine 9(a-f) from the γ-dielectrophile 7 and primary amines 8(a-f)

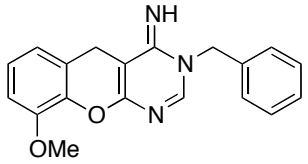




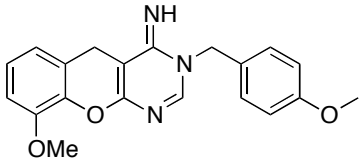
9a:
94% from **7**
34% from **4e**
React. temp. = 50°C



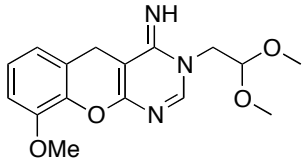
9b:
76% from **7**
27% from **4e**
React. temp. = 75°C



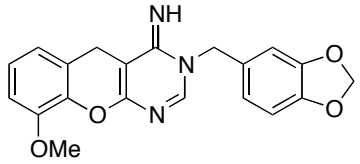
9c:
86% from **7**
31% from **4e**
React. temp. = 120°C



9d:
66% from **7**
24% from **4e**
React. temp. = 120°C



9e:
52% from **7**
19% from **4e**
React. temp. = 120°C



9f:
49% from **7**
18% from **4e**
React. temp. = 120°C

Again.... thanks to microwave !



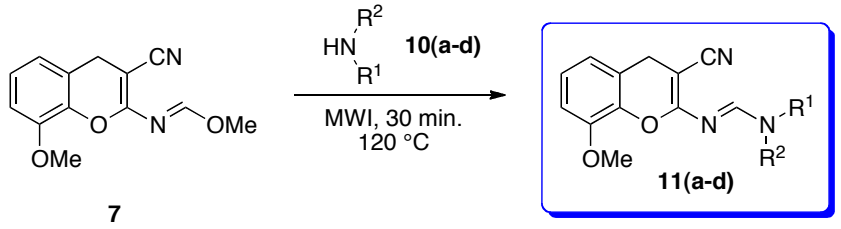
Remarks on microwave reaction conditions:

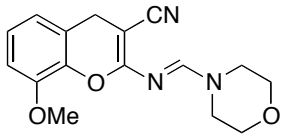
- ✓ Solvent: dry EtOH
- ✓ **8**, only 1 equiv.
- ✓ **9**, insoluble.....separation by simple filtration + recrystallization from EtOH
- ✓ good yields for **9** (49-94%) associated to good overall yields (18-34%)

*Near future..... It's possible to increase the molecular diversity with another γ-dielectrophiles **7** and primary amines **8** !*

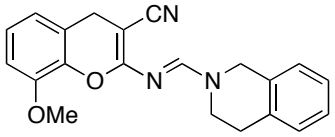
A. Bouattour et al., *Synthesis* **2017**, *49*, 3768-3774

Part 2.2: Microwave synthesis of formamidine derivatives 11(a-d) from the γ-dielectrophile 7 and secondary amines 10(a-d)

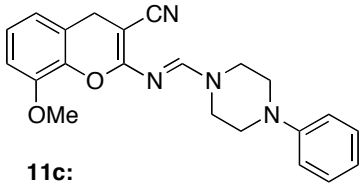




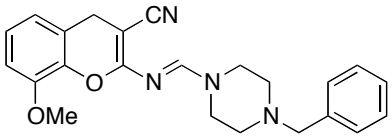
11a:
85% from **7**
31% from **4e**



11b:
63% from **7**
23% from **4e**



11c:
70% from **7**
25% from **4e**



11d:
73% from **7**
26% from **4e**



Remarks on microwave reaction conditions:

- ✓ Solvent: dry EtOH
- ✓ **10**, only 1.1 equiv.
- ✓ **11**, insoluble…..separation by simple filtration + recrystallization from EtOH
- ✓ high yields for **11** (63-85%) associated to good overall yields (23-31%)

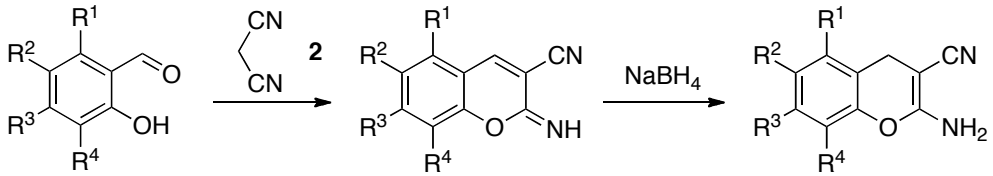
Again…. thanks to microwave !

Near future…..
*It's also possible to increase the molecular diversity on formamidines **11** with another γ-dielectrophiles **7** and secondary amines **10** !*

A. Bouattour et al., *Synthesis* **2017**, *49*, 3768-3774

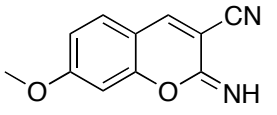
Conclusions

- ✓ A practical and efficient approach to 2-amino-4*H*-benzopyran 4(a-f) without substituent in C-5 position using simple reaction conditions was developed

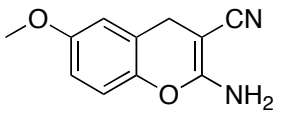


1(a-f) 3(a-f) 4(a-f)

- ✓ Two new submicromolar protein inhibitors of CLK1 were identified

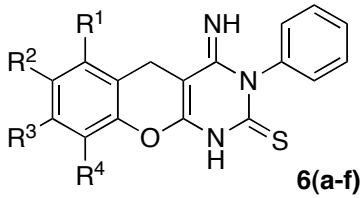


3d
CLK1: IC_{50} 0.25 μM



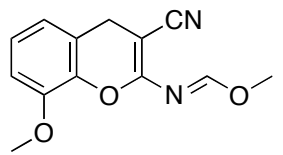
4c
CLK1: IC_{50} 0.9 μM

- ✓ Microwave appeared as a real and practical tool for the synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f) in short reaction time with good yields



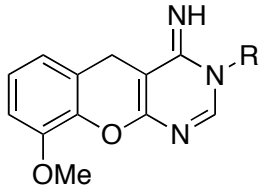
6(a-f)

- ✓ A cheap and practical synthesis of the γ-dielectrophile 7 was realized under microwave with only 0.5 mol% of AcOH



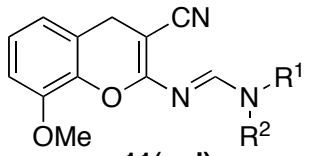
7

- ✓ From the γ-dielectrophile 7, access to new 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine 9(a-f) was realized sucessfully under microwave irradiation



9(a-f)

- ✓ Again with the γ-dielectrophile 7 under microwave irradiation with secondary access, a practical synthesis of formamidine derivatives 11(a-d) was realized in 30 min.



11(a-d)

Extensions

- ✓ Amplify the exploration of molecular diversity on CLK1 inhibitors 3d and 4c
- ✓ Extend the synthesis of other γ-dielectrophile 7 under microwave for the synthesis of new 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine 9 and formamidine derivatives 11
- ✓ Evaluate the effects of 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine 9(a-f) and formamidine derivatives 11(a-d) on the catalytic activity of protein kinases and their antiproliferative activity on tumor cell lines
- ✓ Explore the chemical reactivity of NH_2 group of 2-amino-4*H*-benzopyran 4 with new electrophile for the synthesis of new and original 4*H*-benzopyran (or 4*H*-chromen) derivatives

Acknowledgments

✓ Financial support (€) for people (JPB, LP) of UR1:

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Cancéropôle Grand ouest

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