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3rd International Electronic Conference on Medicinal Chemistry

1-30 November 2017

chaired by Dr. Jean Jacques Vanden Eynde

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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

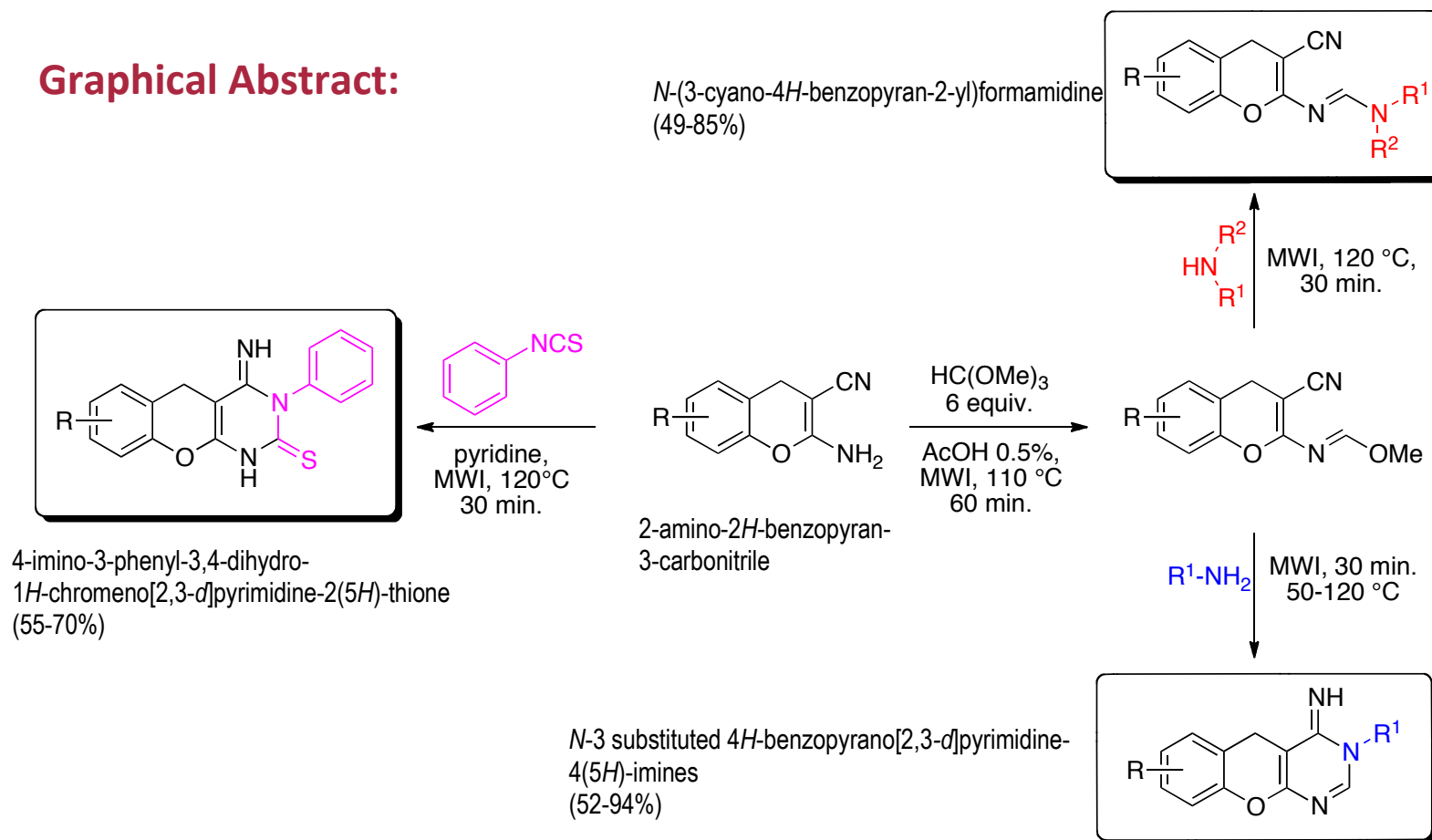
- ¹ Institut des Sciences Chimiques de Rennes, ISCR UMR 6226, groupe CORINT, Université de Rennes 1, Bât. 10A, Campus de Beaulieu, 263 Avenue du Général Leclerc, CS 74205, 35042 Rennes Cedex (France)
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Reactivity under microwave irradiation of 2-amino 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, melanoma, and cancers of liver, colon, breast, prostate, lung, and kidney) and also, *in vitro* Serine/Threonine protein kinase inhibition assays (*Hs*CDK5-p25, GSK3 α/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.



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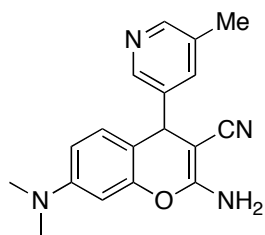
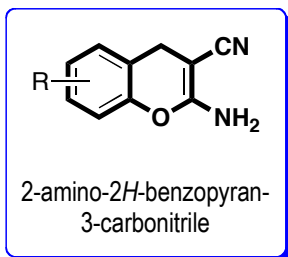
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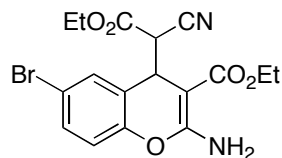
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The 2-amino-4H-benzopyran scaffold.....an interesting structure open to bioactive derivatives !

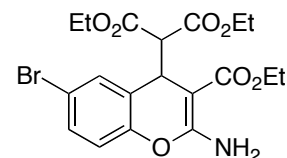
Introduction



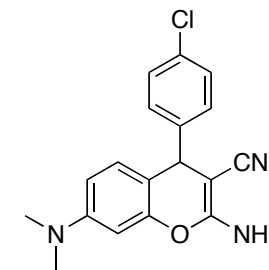
MX58151
anticancer agent
J. Med. Chem. **2008**, 51, 417



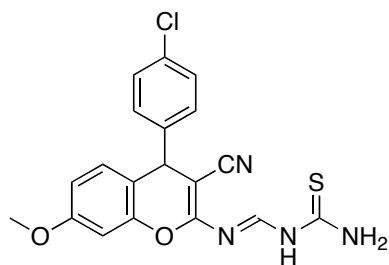
HA 14-1
Bcl-2 inhibitor
Tetrahedron **2009**, 65, 10149
Leukemia Res. **2007**, 31, 859



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

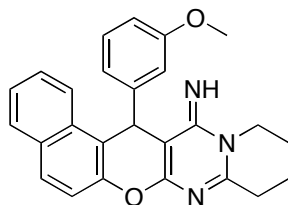


compound 3a
anticancer activity
MCF cell line IC₅₀ 19.7 μM
Eur. J. Med. Chem. **2011**, 46, 765



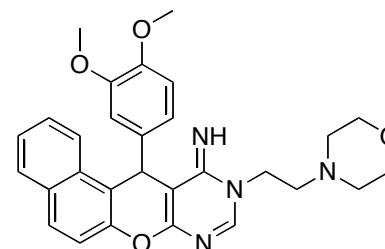
(*R, S*)-*N'*-(4-(4-chlorophenyl)-3-cyano-7-methoxy-4H-chromen-2-yl)-*N*-carbamoylformamimidamide

MCF7 cell line IC₅₀ 8 nM
Der Pharm. Chem. **2012**, 4, 1653

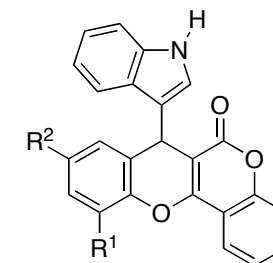


15-(3-methoxyphenyl)-9,11,12,15-tetrahydro-10H-14H-benzo[5,6]chromeno[2,3-d]pyrido[1,2-a]pyrimidine-14-imine

Aβ₁₋₄₂ aggregation inhibitor
hAChE IC₅₀ 58 nM & hBuChE IC₅₀ 302 nM
ChemMedChem **2016**, 11, 1318



NPSR antagonist
cAMP IC₅₀ 4.87 μM
ACS Chem Neurosci. **2010**, 1, 559



7-(1H-indol-3-yl)-6H,7H-chromeno[4,3-b]chromen-6-one
R¹ = H, Br, I
R² = H, Br, I, NO₂, MeO
anti HIV activity
Synlett **2015**, 26, 1969



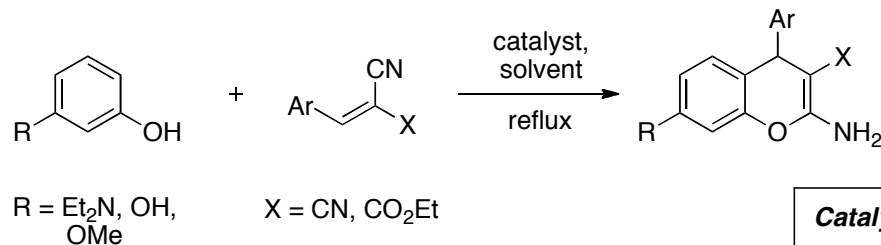
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The usual methods to build the 2-amino-4H-benzopyran derivatives by Michaël addition and cyclisation

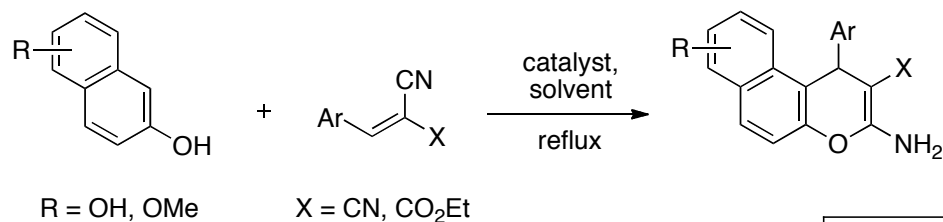


with always substituent in position C-4 !

Catalyst & solvent used:

- piperidine, EtOH: *Eur. J. Med. Chem.* **2011**, 46, 765
- Fe₂O₃, magnetic nanoparticles, H₂O: *Phosphorus, Sulf. and Silicon* **2014**, 189, 1
- K₂CO₃, EtOH: *J. Chem. Pharm. Res.* **2009**, 1, 213
- InCl₃, H₂O/EtOH: *Tetrahedron Lett.* **2007**, 48, 6785
-))) irradiation, Fe₃O₄-chitosan MNPs, H₂O: *Ultrason. Sonochem.* **2015**, 22, 341

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



Catalyst & solvent used:

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



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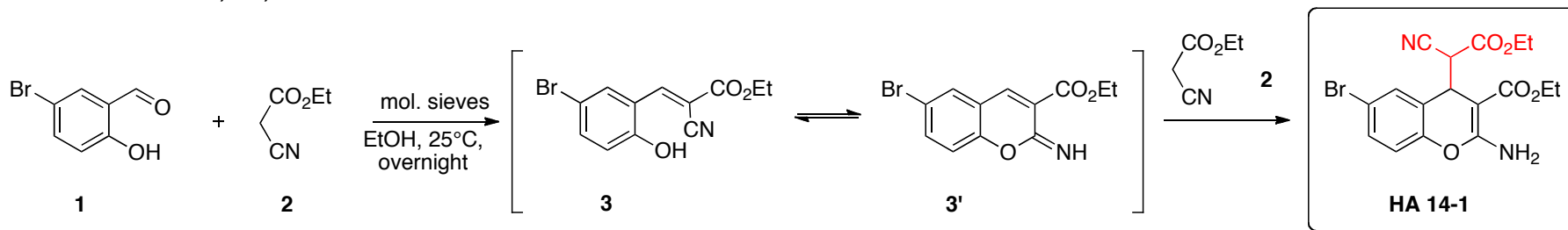
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Other methods to build the 2-amino-4*H*-benzopyran derivatives

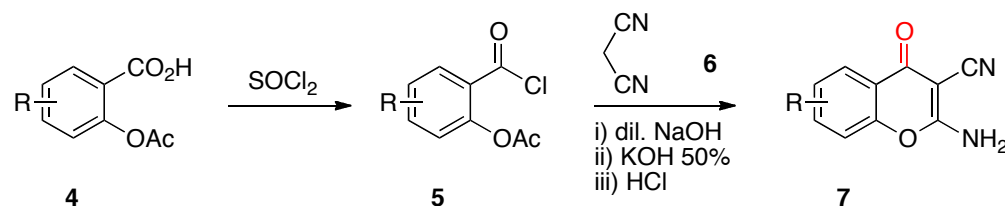
Tetrahedron Lett. **2008**, *49*, 3276-78



via iminocoumarin **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



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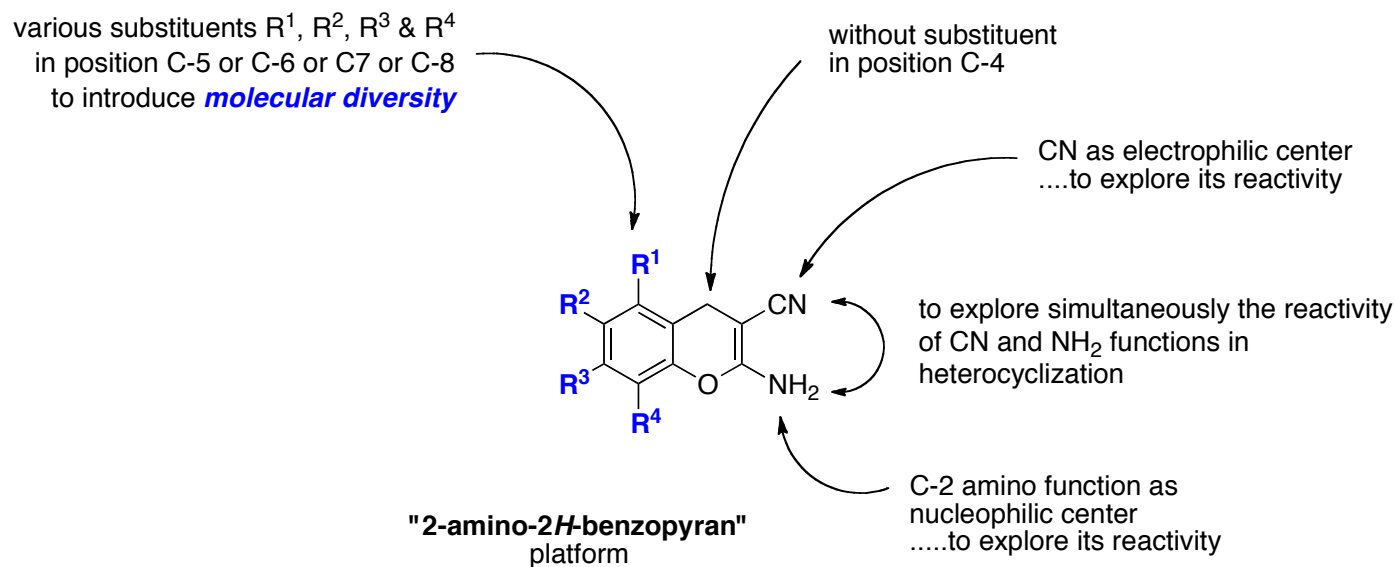
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Our major interests on 2-amino-4H-benzopyran platform:

1. To build the 2-amino-4H-benzopyran platform *via iminocoumarin chemistry* using simple methods of organic chemistry,
2. To prepare original compounds after heterocyclization between CN and NH₂ functions,
3. To explore introduction of amidine function using NH₂ 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-4H-benzopyran scaffold and their derivatives....

As potential protein kinase inhibitors (on 6 selected PKs)

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.

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

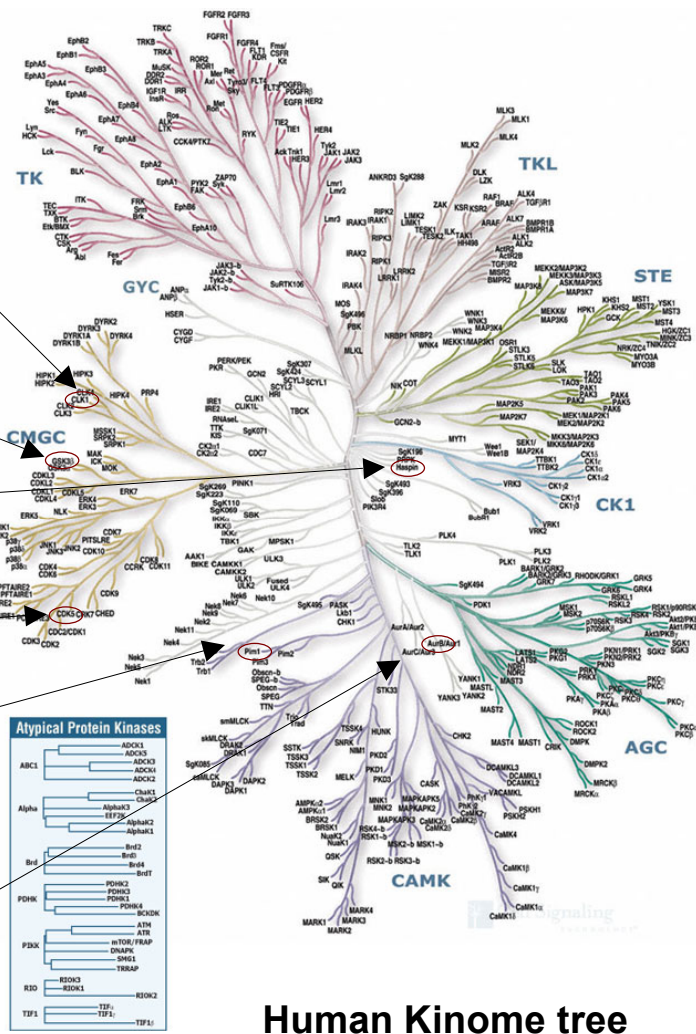
GSK3 α/β : is a serine/threonine protein kinase that mediates the addition of phosphate molecules onto serine and threonine amino acid residues

HsHaspin: is a serine/threonine protein kinase encoded by the GSG2 gene

HsCDK5-p25: (Cyclin-dependant kinase 5) is an enzyme encoded by the CDK5 gene.

HsPim1: Proto-oncogene serine/threonine protein kinase encoded by the Pim1 gene

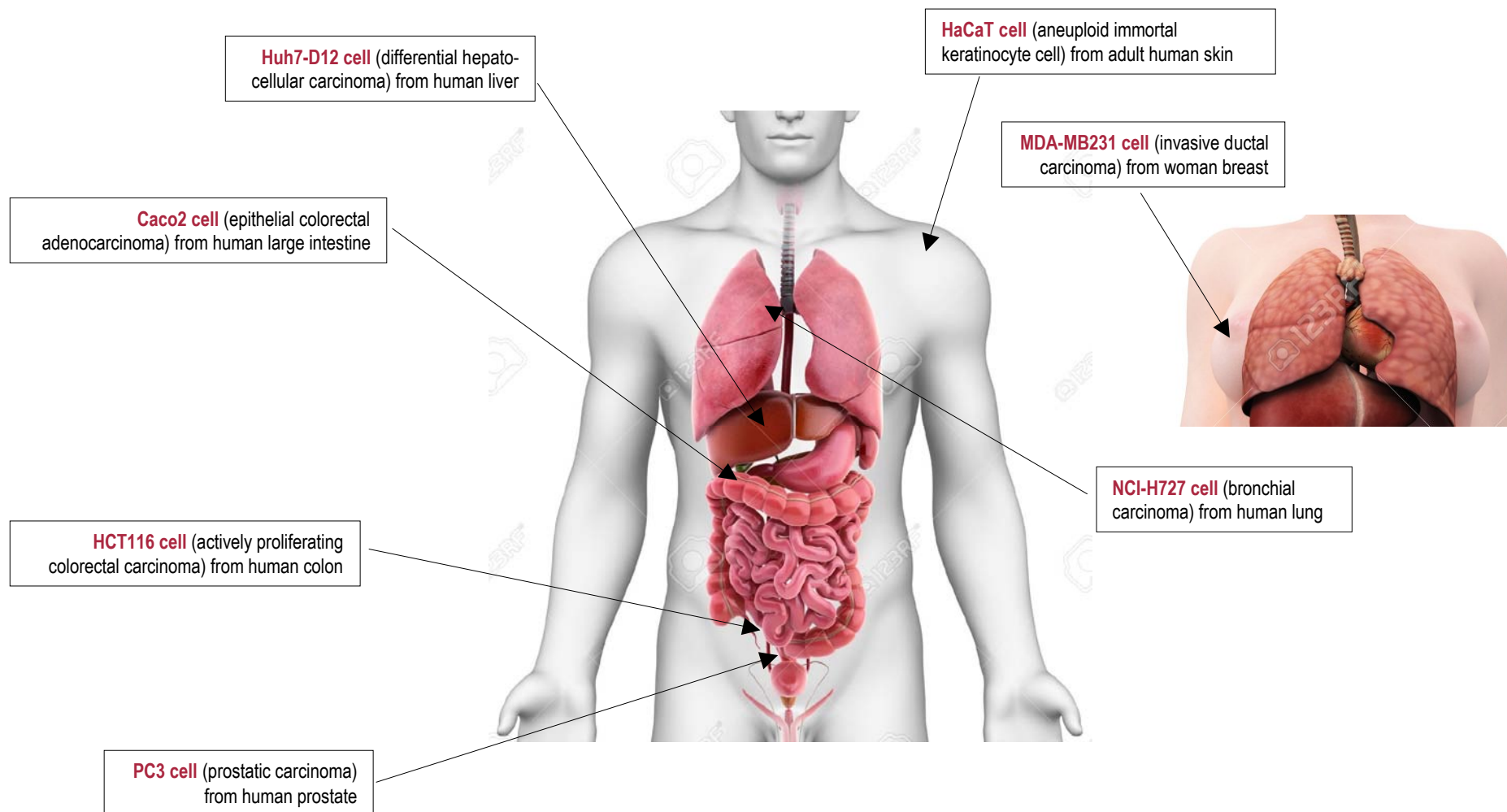
HsAurora B: is a protein that functions in the attachment of the mitotic spindle to the centromere



Human Kinome tree

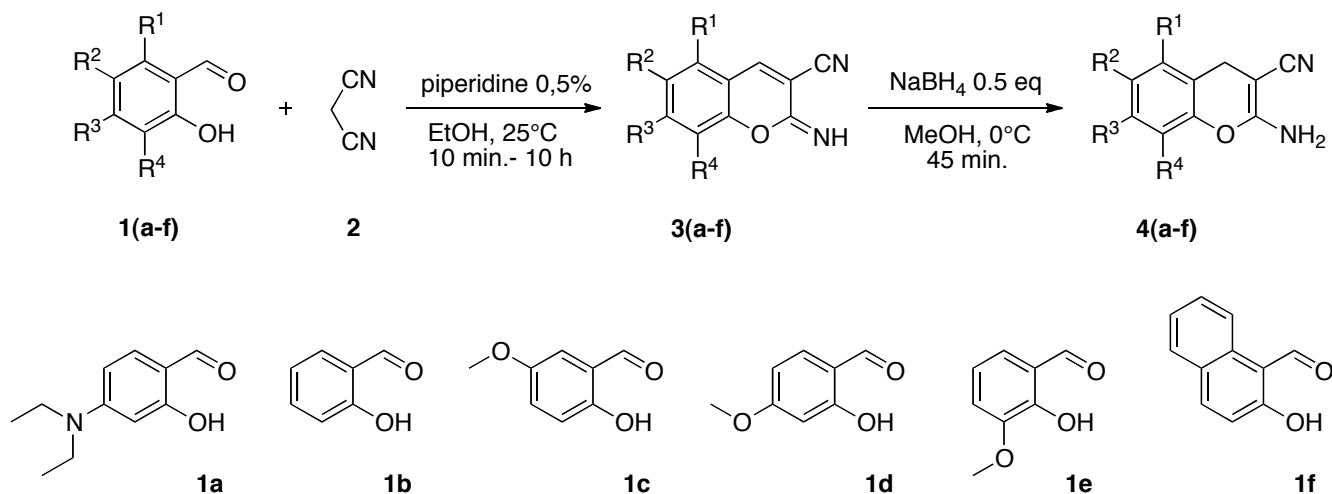
Our interest on 2-amino-4H-benzopyran scaffold and their derivatives....

Against selected tumoral cell lines



Results and discussion

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



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



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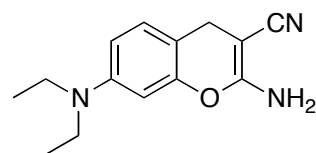
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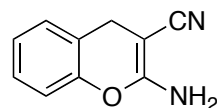
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Part 1.1: Results for the synthesis of 2-amino-4H-benzopyran 4(a-f)

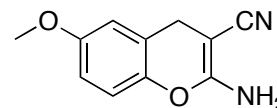
Access to 2-amino-4H-benzopyran platform is very easy !



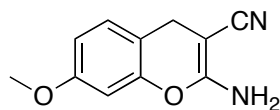
4a: 70% yield
 $\delta_{\text{H-4}} = 3.25$ ppm



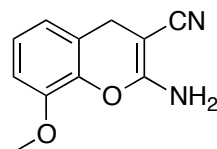
4b: 82% yield
 $\delta_{\text{H-4}} = 3.45$ ppm



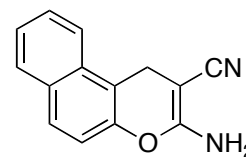
4c: 50% yield
 $\delta_{\text{H-4}} = 3.42$ ppm



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



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



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

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

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

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



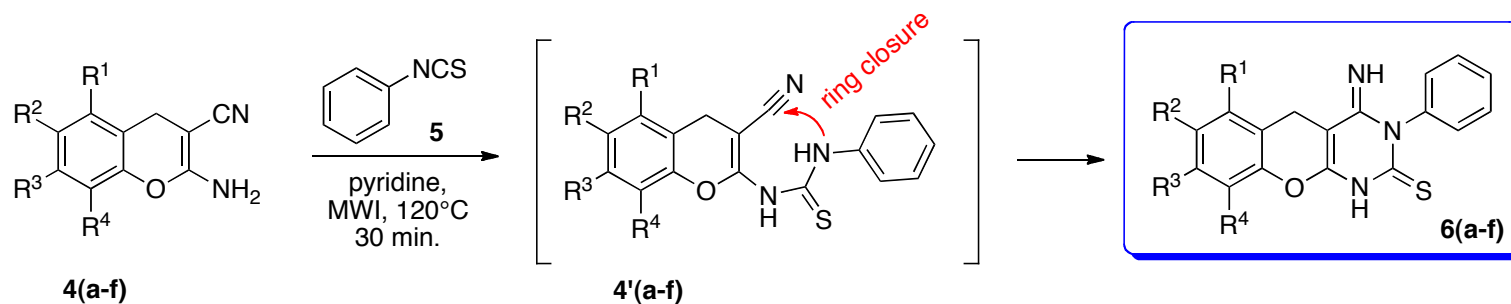
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Part 1.2: Transformation of 2-amino-4*H*-benzopyran 4(a-f) into 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f)



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



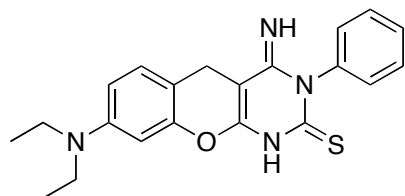
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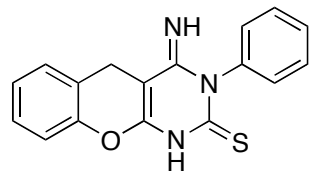


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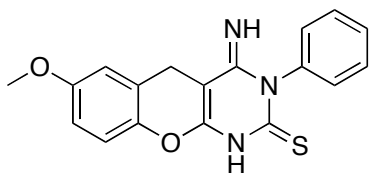
Part 1.3: Results for the preparation of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f)



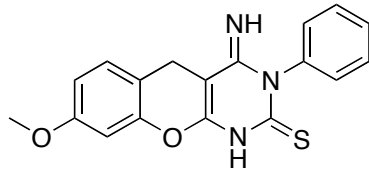
6a: 60% yield
25% overall yield
 $\delta_{H-4} = 3.56$ ppm



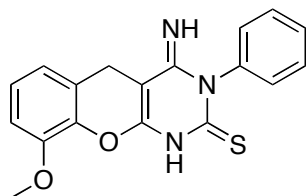
6b: 67% yield
44% overall yield
 $\delta_{H-4} = 3.73$ ppm



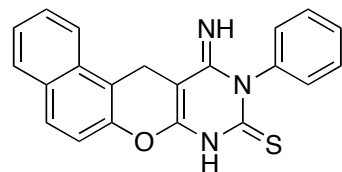
6c: 70% yield
21% overall yield
 $\delta_{H-4} = 3.72$ ppm



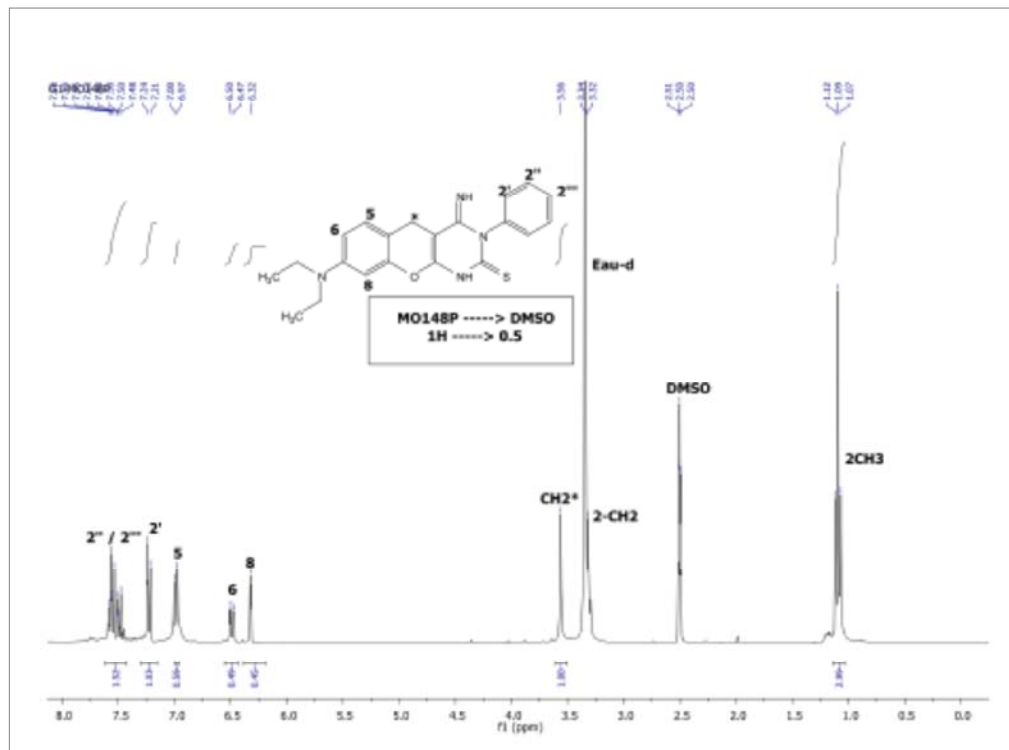
6d: 65% yield
41% overall yield
 $\delta_{H-4} = 3.64$ ppm



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



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



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

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



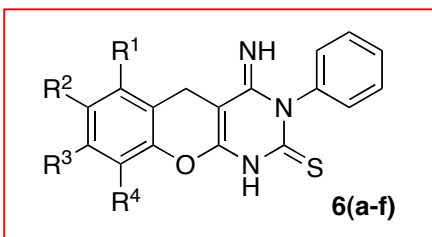
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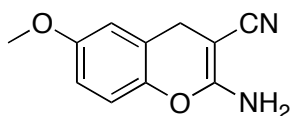
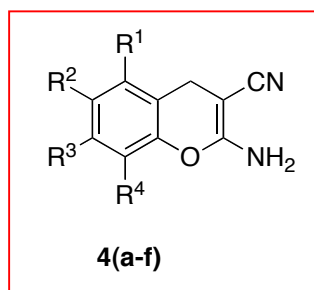


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Part 1.4: Effects of compounds 3(a-f), 4(a-f) and 6(a-f) on the catalytic activity of protein kinases and antiproliferative activity on tumor cell lines

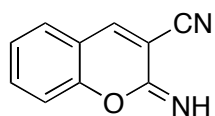


None of compounds 6(a-f) presented a significant toxicity ($IC_{50} > 25 \mu\text{M}$) on tumoral cell lines (Huh7 D12, Caco2, MDA-MB231, HCT 116, PC3, NCI-H727 and HaCaT) and no inhibitory activity on protein kinases (*Hs*CDK5-p25, GSK3 α/β , CLK1, *Hs*Haspin, *Hs*Pim1 and *Hs*Aurora B).

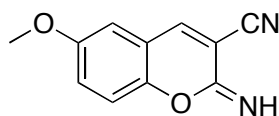


4c
CLK1: IC_{50} 0.9 μM

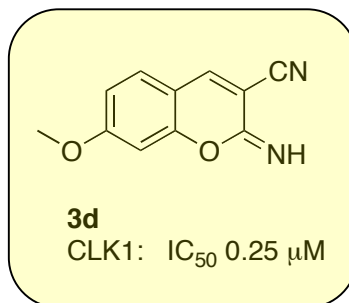
Only the 2-amino-4H-benzopyran-3-carbonitrile 4c inhibited selectively the protein kinase CLK1



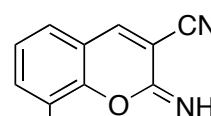
3b
Huh7: IC_{50} 2 μM
Caco2: IC_{50} 6 μM



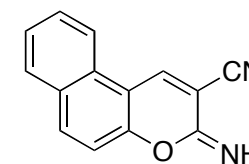
3c
Caco2: IC_{50} 6 μM
CLK1: IC_{50} 1.5 μM



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



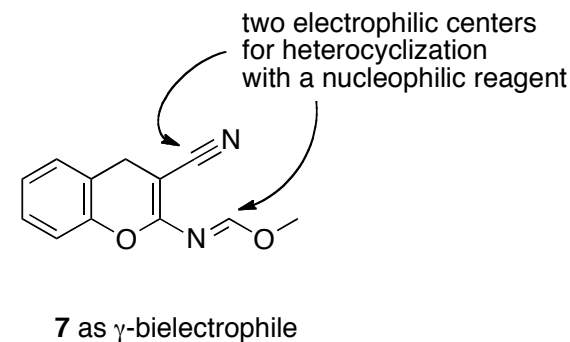
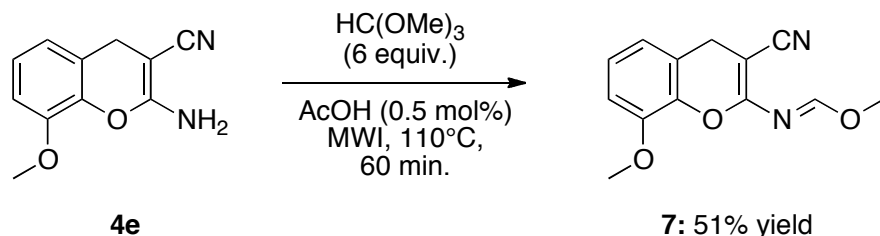
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Part 2: Interest of **microwave irradiation** for transformation of 2-amino-4H-benzopyran 4e into γ -biselectrophile methyl methanimidate 7



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



✓ 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



Thanks to microwave !



Monowave 300
(850 Watt)



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



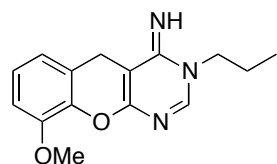
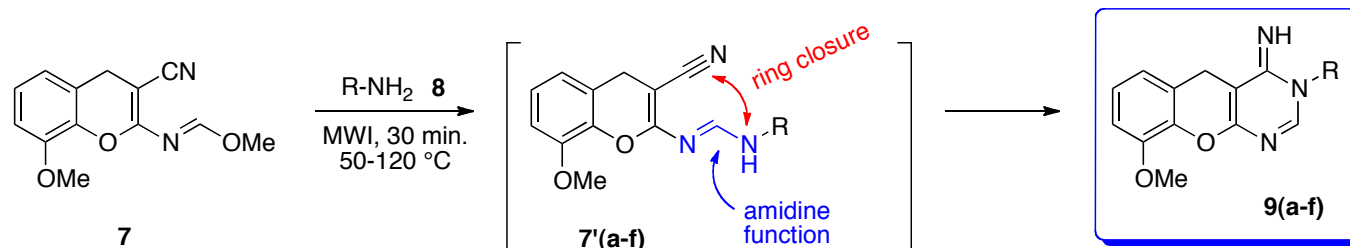
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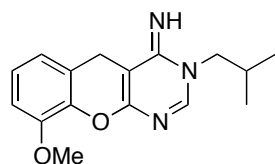


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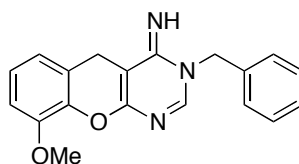
Part 2.1: Microwave synthesis of 4*H*-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine 9(a-f) from the γ -dielectrophile 7 and primary amines 8(a-f)



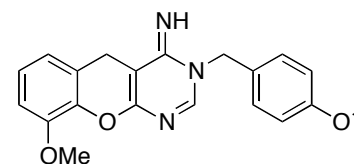
9a:
94% from 7
34% from 4e
React. temp. = 50°C



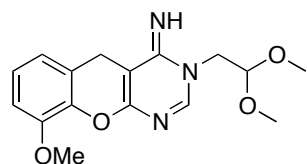
9b:
76% from 7
27% from 4e
React. temp. = 75°C



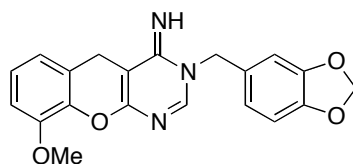
9c:
86% from 7
31% from 4e
React. temp. = 120°C



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



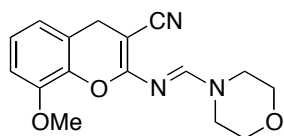
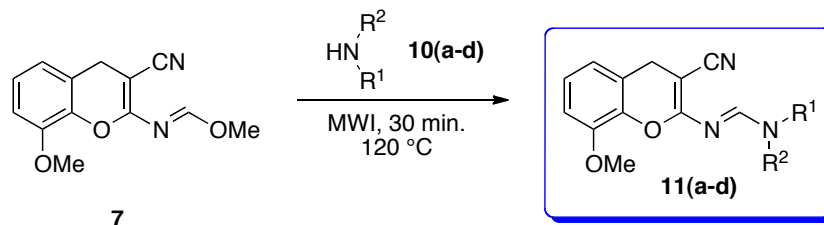
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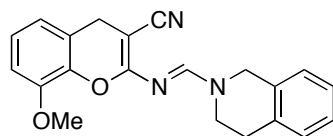


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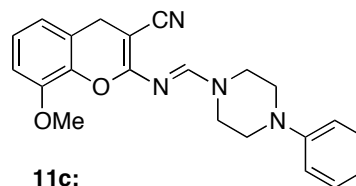
Part 2.2: Microwave synthesis of formamidine derivatives 11(a-d) from the γ -dielectrophile 7 and secondary amines 10(a-d)



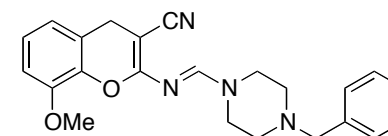
11a:
85% from 7
31% from 4e



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 !

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

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



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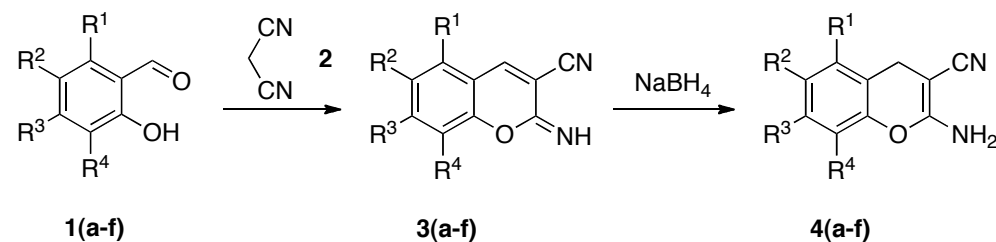
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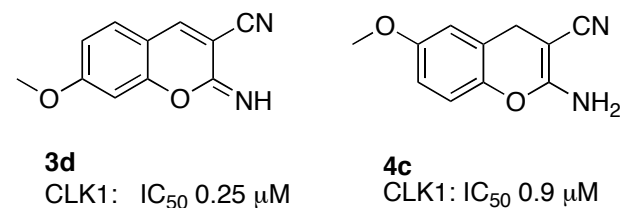
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Conclusions

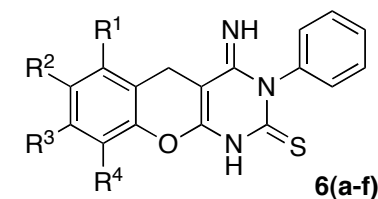
- ✓ A practical and efficient approach to 2-amino-4*H*-benzopyran 4(a-f) without substituent in C-5 position using simple reaction conditions was developed



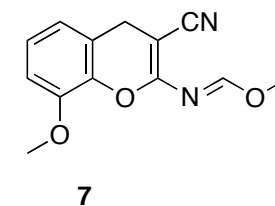
- ✓ Two new submicromolar protein inhibitors of CLK1 were identified



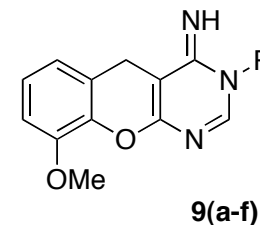
- ✓ Microwave appeared as a real and practical tool for the synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f) in short reaction time with good yields



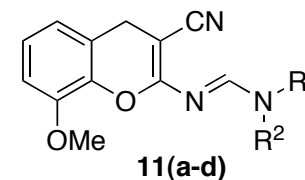
- ✓ A cheap and practical synthesis of the γ -dielectrophile 7 was realized under microwave with only 0.5 mol% of AcOH



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



✓ 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.



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 NH₂ 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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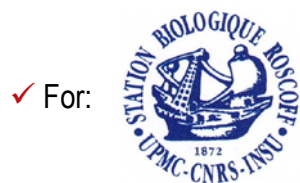
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