Reactivity under microwave irradiation of 2-amino 4H-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)
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)

* Corresponding author: jean-pierre.bazureau@univ-rennes1.fr
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Graphical Abstract:

- 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione (55-70%)
- 2-amino-2H-benzopyran-3-carbonitrile
- N-3 substituted 4H-benzopyran[2,3-d]pyrimidine-4(5H)-imines (52-94%)

$N$-(3-cyano-4H-benzopyran-2-yl)formamidine (49-85%)

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**Abstract:** The interest of 4H-chromenes (or 4H-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-2H-benzopyran-3-carbonitrile platform, it was possible to built easily (20 min.) a new class of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thiones (55-70%) under microwave at 120°C in pyridine medium. Treatment of the amino 4H-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 4H-benzopyrano[2,3-d]pyrimidine-4(5H)-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-2H-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-2H-benzopyran-3-carbonitrile

MX58151
anticancer agent

HA 14-1
Bcl-2 inhibitor
Tetrahedron 2009, 65, 10149

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

compounds 3a
anticancer activity
MCF cell line IC50 19.7 µM

(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

3rd International Electronic Conference on Medicinal Chemistry
1-30 November 2017
The usual methods to build the 2-amino-4H-benzopyran derivatives by Michaël addition and cyclisation

\[
\begin{align*}
\text{R = Et}_2\text{N, OH, OMe} & \quad \text{X = CN, CO}_2\text{Et} \\
\text{R} & \quad \text{Ar} \\
\text{catalyst, solvent} & \\
\text{reflux} &
\end{align*}
\]

with always substituent in position C-4 !

Catalyst & solvent used:
- Fe\textsubscript{2}O\textsubscript{3}, magnetic nanoparticles, H\textsubscript{2}O: *Phosphorus, Sulf. and Silicon* 2014, 189, 1
- K\textsubscript{2}CO\textsubscript{3}, EtOH: *J. Chem. Pharm. Res.* 2009, 1, 213
- InCl\textsubscript{3}, H\textsubscript{2}O/EtOH: *Tetrahedron Lett.* 2007, 48, 6785
- \(\text{[((irradiation, Fe}_3\text{O}_4\text{-chitosan MNPs, H}_2\text{O: Ultrason. Sonochem. 2015, 22, 341}}

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

\[
\begin{align*}
\text{R = OH, OMe} & \quad \text{X = CN, CO}_2\text{Et} \\
\text{R} & \quad \text{Ar} \\
\text{catalyst, solvent} & \\
\text{reflux} &
\end{align*}
\]

Catalyst & solvent used:
Other methods to build the 2-amino-4H-benzopyran derivatives

Tetrahedron Lett. 2008, 49, 3276-78

\[
\begin{align*}
\text{Br} & \quad \text{CO}_2\text{Et} \\
\text{OH} & \quad \text{CN} \\
\text{Br} & \quad \text{CN} & \quad \text{Br} & \quad \text{NH}_2
\end{align*}
\]

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

\[
\begin{align*}
\text{R} & \quad \text{SOCl}_2 \\
\text{OAc} & \quad \text{CN} & \quad \text{CN} & \quad \text{NH}_2
\end{align*}
\]

with C=O in position C-4
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.
Our interest on 2-amino-4H-benzopyran scaffold and their derivatives...

Against selected tumoral cell lines

- **Huh7-D12 cell** (differential hepatocellular carcinoma) from human liver
- **Caco2 cell** (epithelial colorectal adenocarcinoma) from human large intestine
- **HCT116 cell** (actively proliferating colorectal carcinoma) from human colon
- **PC3 cell** (prostatic carcinoma) from human prostate
- **HaCaT cell** (aneuploid immortal keratinocyte cell) from adult human skin
- **MDA-MB231 cell** (invasive ductal carcinoma) from woman breast
- **NCI-H727 cell** (bronchial carcinoma) from human lung
Results and discussion

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

![Chemical structures and reactions]

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-4H-benzopyran 4(a-f)

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

Good yields for reduction with NaBH₄ in the 2nd step: 50-95%

Characteristic signal for ¹H NMR in DMSO-d₆ solution: CH₂ in H-4 position, 3.25 < δ_H-4 < 3.77 ppm

A. Bouattour et al., Arkivoc 2017, iv, 291-302
Part 1.2: Transformation of 2-amino-4H-benzopyran 4(a-f) into 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-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 !

A. Bouattour et al., Arkivoc 2017, iv, 291-302
Part 1.3: Results for the preparation of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione 6(a-f)

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

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

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

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

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

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

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

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

None of compounds 6(a-f) presented a significant toxicity (IC\textsubscript{50} > 25 \(\mu\)M) on tumoral cell lines (Huh7 D12, Caco2, MDA-MB231, HCT 116, PC3, NCI-H727 and HaCaT) and no inhibitory activity on protein kinases (HsCDK5-p25, GSK3\(\alpha/\beta\), CLK1, HsHaspin, HsPim1 and HsAurora B).

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

Selective and submicromolar inhibitor of protein kinase CLK1
Part 2: Interest of microwave irradiation for transformation of 2-amino-4H-benzopyran 4e into \( \gamma \)-bilectrophile methyl methanimidate 7

\[
\begin{align*}
4e & \xrightarrow{\text{HC(OMe)}_3 \text{ (6 equiv.)}} \text{AcOH (0.5 mol%) MWI, 110°C, 60 min.} & 7: 51\% \text{ yield} \\
\end{align*}
\]

- 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 !

A. Bouattour et al., *Synthesis* 2017, 49, 3768-3774
Part 2.1: Microwave synthesis of 4H-benzopyrano[2,3-d]pyrimidine-4(5H)-imine 9(a-f) from the γ-dielectrophile 7 and primary amines 8(a-f)

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

![Diagram of the microwave synthesis reaction]

**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%)

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!
Conclusions

✓ A practical and efficient approach to 2-amino-4H-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-1H-chromeno[2,3-d]pyrimidine-2(5H)-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 4H-benzopyrano[2,3-d]pyrimidine-4(5H)-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 4H-benzopyrano[2,3-d]pyrimidine-4(5H)-imine 9 and formamidine derivatives 11
- Evaluate the effects of 4H-benzopyrano[2,3-d]pyrimidine-4(5H)-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-4H-benzopyran 4 with new electrophile for the synthesis of new and original 4H-benzopyran (or 4H-chromen) derivatives
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