



# Practical approach to N-benzyl derivatives of 2-amino-8-methoxy-4H-chromene-3-carbonitrile by reductive amination: Exploration of their effects against protein kinases and in silico ADME profiling

Marwa Dhiabi, Ali Bouattour, Mehdi Fakhfakh, Souhir Abid, Ludovic Paquin, Thomas Robert, Stéphane Bach, Jean-Pierre Bazureau, Houcine Ammar

## ► To cite this version:

Marwa Dhiabi, Ali Bouattour, Mehdi Fakhfakh, Souhir Abid, Ludovic Paquin, et al.. Practical approach to N-benzyl derivatives of 2-amino-8-methoxy-4H-chromene-3-carbonitrile by reductive amination: Exploration of their effects against protein kinases and in silico ADME profiling. Journal of Molecular Structure, 2023, 1274 (Part 1), pp.134319. 10.1016/j.molstruc.2022.134319 . hal-03867314

**HAL Id: hal-03867314**

**<https://hal.science/hal-03867314>**

Submitted on 15 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Practical approach to *N*-benzyl derivatives of 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile by reductive amination: exploration of their effects against protein kinases and *in silico* ADME profiling

Marwa Dhiabi <sup>a,b</sup>, Ali Bouattour <sup>a</sup>, Mehdi Fakhfakh <sup>a</sup>, Souhir Abid <sup>c</sup>, Ludovic Paquin <sup>b,\*</sup>, Thomas Robert <sup>d,e</sup>, Stephane Bach <sup>d,e</sup>, Jean-Pierre Bazureau <sup>b,\*</sup> and Houcine Ammar <sup>a</sup>

<sup>a</sup> *Laboratoire de Chimie Appliquée: Hétérocycles, Corps Gras et Polymères. Faculté des Sciences de Sfax. Université de Sfax, Route Soukra, BP 1171, 3000 Sfax, Tunisie.*

<sup>b</sup> *Institut des Sciences Chimiques de Rennes ISCR UMR CNRS 6226, Université de Rennes 1, Bât. 10A, Campus de Beaulieu, CS 74205, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France.*

<sup>c</sup> *Al-Jouf University, Department of Chemistry (Science and Arts): Al Qurayat, Al Jawf, Saudi Arabia.*

<sup>d</sup> *Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, Place George Tessier, BP 74, 29680 Roscoff, France.*

<sup>e</sup> *Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, Place George Plessier, BP 74, 29680 Roscoff, France.*

Corresponding author : [jean-pierre.bazureau@univ-rennes1.fr](mailto:jean-pierre.bazureau@univ-rennes1.fr), phone: +33 223 236 603

Bazureau  <https://orcid.org/0000-0003-3053-3425>

Paquin  <https://orcid.org/0000-0001-8518-5363>

Bach  <https://orcid.org/0000-0002-7186-7483>

## Abstract:

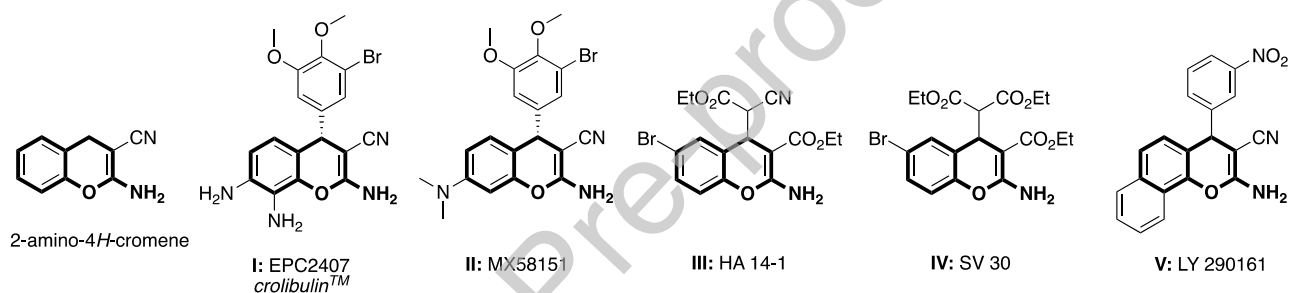
The synthesis of 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitriles **7(a-e)** has been realized in four steps, *via* reductive amination from the 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile **4** as key intermediate platform with *para*- and *meta*-substituted benzaldehydes **5(a-e)**, in good overall yields. The physicochemical properties of **7(a-e)** and also their aldimines **6(a-e)** precursors have been determined using the Swiss ADME server platform according to the Lipinski's descriptors. Biological assays with a panel of six protein kinases such as *Hs*CDK5-p25, *Hs*CDK9/cyclin T, *Hs*Pim1, *Hs*Haspin, *Ssc*GSK-3α/β and *Ssc*CK1δ/ε showed that aldimines **6(a,b)** and **6e** are potentially interesting because they showed good percentage of residual activities against *Hs*Pim1 and *Hs*Haspin.

**Key words:** 2-amino-4*H*-chromene; reductive amination; *N*-benzylation; Lipinski's descriptors, protein kinase, Pim1 inhibitor, Haspin inhibitor

Journal Pre-proof

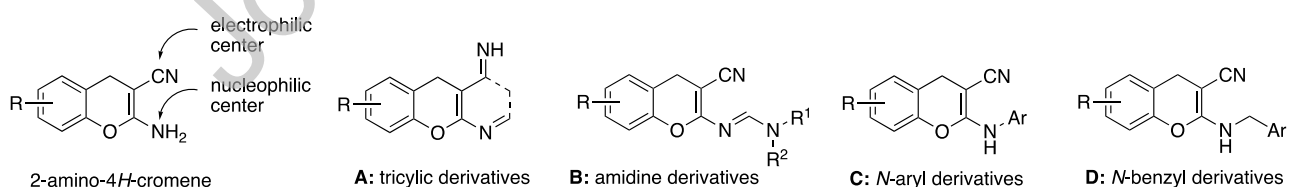
## 1 Introduction

Chromenes and benzochromenes are notorious because many derivatives such as 2-amino-4*H*-chromene-3-carbonitriles (or their 3-carboesters analogues) exhibits a wide range of biological and pharmacological activities<sup>1</sup>. For examples in Figure 1, EPC 2407 (I) or crolibulin™ was identified in clinical phase I/II trials as drug-candidate<sup>2</sup> for the treatment of advanced solid tumors on anaplastic thyroid cancer (ATC). During the randomized phase II trial (NTC 01240590)<sup>3</sup>, the antitumoral activity of crolibulin™ plus cisplatin was compared to cisplatin alone on 40 enrolled patients for treatment of ATC. The 7-dimethylamino derivative of crolibulin™ or MX5815 (II)<sup>4</sup> was evaluated as apoptosis inducer (EC<sub>50</sub> 50 nM) and inhibited cell growth of TD47D breast cancer cells<sup>5</sup> with a GI of 37 nM. Among the 2-amino-4*H*-chromene derivatives, HA14-1 (III) was identified as a small non-peptidic ligand of Bcl-2 protein<sup>6</sup> using a *de novo* computer-aided design strategy based on the predicted structure of Bcl-2 protein<sup>7</sup>. The diester derivative SV30 (IV) which is an analogue of pro-apoptotic molecule HA14-1 (III) was associated to lipid nanocapsules (LNCs) on F98 cells to improve its biological activities<sup>8</sup>. For the naphthopyran derivative LY 290161 (V)<sup>9</sup>, it showed antiproliferative activity on microtubulin binding, particularly in the G2/M phase of the cell cycle<sup>10</sup>.



**Figure 1:** Bioactive 2-amino-4*H*-chromene derivatives reported in literature

Due to the potential pharmacological interest of the 2-amino-4*H*-chromene-3-carbonitrile platform (Figure 1), we were motivated in our laboratory to explore the chemical reactivities of the carbonitrile function (electrophilic center) and the 2-amino group (nucleophilic center) for the construction of new heterocycle derivatives in combination to exploration of biological activities, such as their antiproliferative activities on tumor cell lines or inhibition activities of protein kinases (PKs) in a preliminary approach.



**Figure 2:** Possibilities of molecular diversity offered by the 2-amino-4*H*-chromene-3-carbonitrile platform

In previous works, we showed that this platform (Figure 2) offers the possibilities to built tricyclic compounds (A)<sup>11</sup>, amidines (B)<sup>12</sup> and *N*-aryl derivatives (C) by *trans*-imination of 2-imino-precursors<sup>13</sup>. In continuation of these works, we decided to explore now the construction of *N*-benzyl derivatives (D) of 2-amino-4*H*-chromene-3-carbonitrile, which are potentially the mono *N*-alkylated compounds of this platform using various benzyl halides. *In silico* analysis of the physicochemical properties (log P, partition coefficient; log S, water solubility; tPSA, topological polar surface area; HBA and HBD, hydrogen bond acceptor and donor) will be also realized. Evaluation of their protein kinases (PKs) inhibition activities will be also evaluated in order to detect a potential PK inhibitor.

## 2 Material and methods

### 2.1 Chemistry

#### 2.1.1 General information:

All the required chemical reagents and solvents for this work were purchased from Merck Sigma-Aldrich, Acros Organics Thermo Fisher Scientific France, VWR France and were used without further purification. Melting points were determined on a Kofler melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was accomplished on 0.2-mm precoated plates of silica gel 60 F-254 (Merck) and visualization was made with ultraviolet light (254 and 312 nm). Solvents were evaporated with a BUCHI rotary evaporator. Infrared (IR) spectra were recorded on a Jasco FT-IR 420 spectrophotometer apparatus using KBr pellets.  $^1\text{H}$  NMR spectra were recorded on BRUKER AC 300 Avance III (300 MHz) spectrometer,  $^{13}\text{C}$  NMR spectra on BRUKER AC 300 Avance III (75 MHz) spectrometer using respectively  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  with tetramethylsilane as internal reference. Chemical shifts are expressed in parts per million downfield (ppm). Data are given in the following order:  $\delta$  value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), and number of protons, and coupling constants  $J$  is given in Hertz. The high-resolution mass spectra (HRMS) were enregistered in positive mode using a direct electrospray infusion (ESI), respectively on a Waters Q-ToF 2 and on a Thermo Fisher Scientific Q-Exactive spectrometers at the CRMPO platform of ScanMAT UAR 2025 CNRS (Bât. 11, Campus Beaulieu, Rennes, France). Elemental analyses (C, H and N) of 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** were performed on a Flash Microanalyzer EA1112 CHNS/O Thermo Electron also in the CRMPO platform.

Copies of the spectra data including IR,  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR, spectra of compounds **6(a-e)** and **7(a-e)** are provided in Supplementary Material.

#### 2.1.2 2-Imino-8-methoxy-2*H*-[1]-benzopyran-3-carbonitrile (**3**)

To a stirred solution (550 rpm) at room temperature of 2-hydroxy-3-methoxybenzaldehyde **1** (1.52 g, 10 mmol) in absolute ethanol (20 mL) was added dropwise commercial propanedinitrile **2** (660.2 mg, 10 mmol) during 15 min in the presence of a catalytic amount of piperidine 0.5% mol. (425 mg, 5 mmol). Vigorous stirring was pursued during 4 hours at room temperature. Then, the reaction mixture was concentrated in a rotary evaporator under reduced pressure for the elimination of volatile, the desired product **3** was collected by filtration on a Büchner funnel (porosity N°4) and rinsed with absolute ethanol (3 x 10 mL). The 2-imino-8-methoxy-2*H*-[1]-benzopyran-3-carbonitrile **3** was dried under a high vacuum ( $10^{-2}$  Torr) at 25°C for 1 hour to give 1.7 g of a yellowish powder (85% yield) and was further used without purification. Mp = 173-175°C.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  : 3.87 (s, 3H,  $\text{OCH}_3$ ); 7.20-7.24 (m, 3H, H-5, H-6, H-7, Ar); 8.34 (s, 1H, H-4, Ar); 8.92 (br s, 1H, NH).

#### 2.1.3 2-Amino-8-methoxy-4*H*-chromene-3-carbonitrile (**4**)

To a stirred and cooled suspension (550 rpm, 0°C) of 2-imino-8-methoxy-2*H*-[1]benzopyran-3-carbonitrile **3** (2.4 g, 12 mmol) in 15 mL of methanol was added portionwise a commercial sodium borohydride 98% (232 mg, 6 mmol). Stirring at 0°C was pursued during 45 min. After stirring, the mixture was poured in 20 mL of deionized water at room temperature without stirring to improve decantation and precipitation. The resulting precipitate was collected by filtration on a Büchner funnel (porosity N°4) and washed with deionized water (3x 10 mL). The desired 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile **4** was dried under high vacuum ( $10^{-2}$  Torr) at 25°C for 1 h that gave a yellowish powder (2.303 g, 95% yield) and was further used without purification. Mp = 152-154 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 1661 (C=C), 2186 (CN), 3332-3456 ( $\text{NH}_2$ ).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$ : 3.43 (s, 2H,  $\text{CH}_2$ ); 3.79 (s, 3H, MeO); 6.72 (d, 1H,  $J$  = 6.2 Hz, H-7, Ar); 6.92 (d, 1H,  $J$  9.2 Hz, H-5, Ar); 7.03 (t, 1H,  $J$  = 9.2 Hz, H-6, Ar); 6.82 (br s, 2H,  $\text{NH}_2$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  : 23.8

(CH<sub>2</sub>, C-4); 48.8 (CH<sub>3</sub>O); 55.6 (C-3, C=); 110.7 (C-7, Ar); 119.8 (CN); 120.4 (C-6, Ar); 121.0 (C-5, Ar); 124.1 (C-10, Ar); 138.5 (C-9, Ar); 147.1 (C-8, Ar); 160.7 (C-2, O-C=, Ar).

#### 2.1.4. General procedure for the synthesis of 2-benzylidenamino-8-methoxy-4H-chromene-3-carbonitrile **6(a-e)**:

In a 50 mL two-necked round-bottomed flask, provided with magnetic stirrer and condenser, containing a solution of 2-amino-8-methoxy-4H-chromene-3-carbonitrile **4** (202 mg, 1 mmol) in 10 ml of absolute ethanol, was added dropwise a solution of various aromatic aldehydes (1 mol) in 5 ml of absolute ethanol in the presence of an equimolar quantity of piperidine (85 mg, 1 mmol) during 15 min. After complete addition, the reaction mixture was refluxed during 5 hours. After cooling down to room temperature, the precipitated material was collected by filtration on a Büchner funnel (porosity N°4), washed with cooled absolute ethanol (2 x 5 mL) and recrystallized from EtOH. Drying under high vacuum (10<sup>-2</sup> Torr) at 25°C for 1 h afforded the desired 2-benzylidenamino-8-methoxy-4H-chromene-3-carbonitrile **6** as a powder and was analyzed by <sup>1</sup>H, <sup>13</sup>C NMR and HRMS.

##### 2.1.4.1 2-[(E)-(Phenylmethylene)amino]-8-methoxy-4H-chromene-3-carbonitrile (**6a**)

Compound **6a** was prepared in 70% yield (203 mg) from benzaldehyde **5a** (106 mg, 1 mmol) according to the standard procedure which gave **6a** as yellowish powder, mp = 206-208 °C. IR (KBr, ν, cm<sup>-1</sup>): 1640 (C=N), 2200 (C≡N). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ : 3.83 (s, 2H, CH<sub>2</sub>); 3.86 (s, 3H, MeO); 6.78 (d, 1H, *J* = 7.4 Hz, H-7, Ar); 7.00 (d, 1H, *J* = 7.5 Hz, H-5, Ar); 7.11 (t, 1H, *J* = 7.9 Hz, H-6, Ar); 7.58 (t, 2H, *J* = 7.3 Hz, H-2', H-4', Ar); 7.64 (t, 1H, *J* = 7.2 Hz, H-3', Ar); 8.02 (d, 2H, *J* = 7.1 Hz, H-1', H-5', Ar); 8.88 (s, 1H, CH=N). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ : 26.5 (CH<sub>2</sub>, C-4); 56.5 (CH<sub>3</sub>O); 82.9 (C-3, C=); 111.8 (C-7, Ar); 118.5 (CN); 119.0 (C-5, Ar); 120.4 (C-6, Ar); 125.6 (C-10, Ar); 129.7 (C-14, C-16, Ar); 130.3 (C-13, C-17, Ar); 133.8 (C-15, Ar); 134.9 (C-12, Ar); 138.9 (C-9, Ar); 148.3 (C-8, Ar); 158.8 (C-2, Ar); 162.4 (C-11). HRMS (ES<sup>+</sup>, MeOH/CH<sub>2</sub>Cl<sub>2</sub> 9:1), *m/z*: 313.0950 found (calculated for C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>Na [M+Na]<sup>+</sup> requires 313.09475).

##### 2.1.4.2 2-[[E)-(4-Dimethylaminophenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile (**6b**)

Compound **6b** was prepared in 52% yield (189 mg) from 4-dimethylaminobenzaldehyde **5b** (149 mg, 1 mmol) according to the standard procedure which gave **6b** as yellowish powder, mp = 238-240 °C. IR (KBr, ν, cm<sup>-1</sup>): 1638 (C=N), 2195 (C≡N). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ : 3.11 (s, 6H, 2-CH<sub>3</sub>); 3.79 (s, 2H, CH<sub>2</sub>); 3.93 (s, 3H, MeO); 6.72 (d, 3H, *J* = 8.9 Hz, H-2', H-4', H-7, Ar); 6.84 (d, 1H, *J* = 7.6 Hz, H-5, Ar); 7.05 (t, 1H, *J* = 7.9 Hz, H-6, Ar); 7.91 (d, 2H, *J* = 8.7 Hz, H-1', H-5', Ar); 8.81 (s, 1H, CH=N). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ : 26.9 (CH<sub>2</sub>, C-4); 40.1 (2-CH<sub>3</sub>); 56.0 (CH<sub>3</sub>O); 78.4 (C-3, C=); 110.7 (C-7, Ar); 111.4 (C-14, C-16, Ar); 119.1 (C-5, Ar); 119.3 (CN); 120.0 (C-6, Ar); 122.0 (C-10, Ar); 124.5 (C-13, C-17, Ar); 132.4 (C-12, Ar); 139.6 (C-9, Ar); 148.3 (C-8, Ar); 153.7 (C-15, Ar); 159.9 (C-2, Ar); 160.8 (C-11). HRMS (ES<sup>+</sup>, MeOH/CH<sub>2</sub>Cl<sub>2</sub> 9:1), *m/z*: 356.1367 found (calculated for C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>Na [M+Na]<sup>+</sup> requires 356.13695).

##### 2.1.4.3 2-[[E)-(4-Methoxyphenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile (**6c**)

Compound **6c** was prepared in 90% yield (288 mg) from 4-methoxybenzaldehyde **5c** (136 mg, 1 mmol) according to the standard procedure which gave **6c** as yellowish powder, mp = 204-206 °C. IR (KBr, ν, cm<sup>-1</sup>): 1645 (C=N), 2201 (C≡N). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ : 3.82 (s, 2H, CH<sub>2</sub>); 3.91 (s, 3H, MeO); 3.94 (s, 3H, MeO); 6.73 (d, 1H, *J* = 7.7 Hz, H-7, Ar); 6.85 (d, 1H, *J* = 8 Hz, H-5, Ar); 7.00 (d, 2H, *J* = 8.7 Hz, H-2', H-4', Ar); 7.07 (t, 1H, *J* = 8.0 Hz, H-6, Ar); 8.01 (d, 2H, *J* = 8.7 Hz, H-1', H-5', Ar); 8.88 (s, 1H, CH=N). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ : 27.0 (CH<sub>2</sub>, C-4); 55.5 (CH<sub>3</sub>, C-15'); 56.1 (CH<sub>3</sub>, C-8'); 81.1 (C-3, C=); 110.8 (C-7, Ar); 114.4 (C-14, C-16, Ar); 118.6 (C-10, Ar); 118.8 (CN); 120.0 (C-5, Ar); 124.7 (C-6, Ar); 128.0 (C-12, Ar); 132.3 (C-13, C-17, Ar); 139.3 (C-9, Ar); 148.3 (C-8, Ar); 159.2 (C-2, Ar); 160.9 (C-11, Ar); 163.7 (C-15). HRMS (ES<sup>+</sup>, MeOH/CH<sub>2</sub>Cl<sub>2</sub> 9:1) *m/z*: 343.1057 found (calculated for C<sub>19</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>Na [M+Na]<sup>+</sup> 343.10531).

#### 2.1.4.4 2-[[*(E)*-(4-Chlorophenyl)methylene]amino]-8-methoxy-4*H*-chromene-3-carbonitrile (**6d**)

Compound **6d** was prepared in 80% yield (260 mg) from 4-chlorobenzaldehyde **5d** (141 mg, 1 mmol) according to the standard procedure which gave **6d** as yellowish powder, mp = 198-200 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 1615(C=N), 2205 (C $\equiv$ N).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.83 (s, 2H,  $\text{CH}_2$ ); 3.93(s, 3H, MeO); 6.72 (d, 1H,  $J$  = 6.9 Hz, H-7, Ar); 6.85 (d, 1H,  $J$  = 7.5 Hz, H-5, Ar); 7.08 (t, 1H,  $J$  = 8.0 Hz, H-6, Ar); 7.47 (d, 2H,  $J$  = 8.5 Hz, H-2', H-4', Ar); 7.98 (d, 2H,  $J$  = 8.5 Hz, H-1', H-5', Ar); 8.89 (s, 1H, CH=N).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 27.1 ( $\text{CH}_2$ , C-4); 56.0 ( $\text{CH}_3\text{O}$ ); 83.4 (C-3, C=); 110.8(C-7, Ar); 118.1 (C-10, Ar); 118.5 (CN); 119.9 (C-5, Ar); 125.0 (C-6, Ar); 129.3(C-14, C-16, Ar); 131.3 (C-13, C-17, Ar); 133.5 (C-12, Ar); 139.2 (C-15, Ar); 139.3 (C-9, Ar); 148.3 (C-8, Ar); 158.6 (C-2, Ar); 160.2 (C-11). HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 347.0556 found  $[\text{M}+\text{Na}]^+$  (calculated for  $\text{C}_{18}\text{H}_{13}\text{N}_2\text{O}_2^{35}\text{ClNa}$   $[\text{M}+\text{Na}]^+$  requires 347.05578).

#### 2.1.4.5 2-[[*(E)*-(3-Methoxyphenyl)methylene]amino]-8-methoxy-4*H*-chromene-3-carbonitrile (**6e**)

Compound **6e** was prepared in 78% yield (250 mg) from 3-methoxybenzaldehyde **5e** (136 mg, 1 mmol) according to the standard procedure which gave **6d** as yellowish powder, mp = 212-214 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 1640(C=N), 2205(C $\equiv$ N).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.84 (s, 2H,  $\text{CH}_2$ ); 3.92 (s, 3H, MeO); 3.94 (s, 3H, MeO); 6.74 (d, 1H,  $J$  = 7.4 Hz, H-7, Ar); 6.86 (d, 1H,  $J$  = 7.9 Hz, H-5, Ar); 7.07 (d, 1H,  $J$  = 7.9 Hz, H-6, Ar); 7.12 (dd, 1H,  $J$  = 9.0, 3 Hz, H-3', Ar); 7.41 (t, 1H,  $J$  = 7.9 Hz, H-4', Ar); 7.58 (d, 1H,  $J$  = 7.5 Hz, H-5', Ar); 7.64 (s, 1H, H-1'); 8.92 (s, 1H, CH=N).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 27.1 ( $\text{CH}_2$ , C-4); 55.5 ( $\text{CH}_3$ , C-14'); 56.1 ( $\text{CH}_3$ , C-8'); 82.8 (C-3, C=); 110.9 (C-13, Ar); 112.9 (C-7, Ar); 118.1 (C-10, Ar); 118.7 (CN); 120.0 (C-5, Ar); 120.0 (C-15, Ar); 123.9 (C-17, Ar); 124.9 (C-6, Ar); 129.8 (C-16, Ar); 136.4 (C-12, Ar); 139.5 (C-9, Ar); 148.3 (C-8, Ar); 158.9 (C-2, Ar); 160.0 (C-14, Ar); 161.7 (C-11). HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 343.1056 found (calculated for  $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_3\text{Na}$   $[\text{M}+\text{Na}]^+$  requires 343.10531).

#### 2.1.5. General procedure for the synthesis of 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)**:

In a 50 mL round-bottomed flask, provided with a magnetic stirrer and condenser, containing a suspension of 2-(benzylidene)amino-8-methoxy-4*H*-chromene-3-carbonitrile **6** (1.2 mmol) dissolved in 12 mL of cooled (0°C) tetrahydrofuran (THF) was added portionwise commercial sodium borohydride 98% (23 mg, 0.6 mmol). Vigorous stirring (500 rpm) at 0°C was pursued during 20 min. The reaction mixture was concentrated in a rotary evaporator under reduced pressure for the elimination of volatile and to the crude residue was added deionized water (15 mL) to ensure precipitation of compound **7**. The insoluble material was collected by filtration on a Büchner funnel (porosity N°4) and washed with deionized water (3x10 mL). Compound **7** was dried under high vacuum ( $10^{-2}$  Torr) at 25°C for 1 h, then recrystallized from absolute ethanol which, gave the desired 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7** as a powder analyzed by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, HRMS and centesimal analysis.

##### 2.1.5.1 2-(Phenylmethyl)amino-8-methoxy-4*H*-chromene-3-carbonitrile (**7a**)

Compound **7a** was prepared in 96% yield (337 mg) from 2-[(*E*)-(phenylmethylene)amino]-8-methoxy-4*H*-chromene-3-carbonitrile **6a** (348 mg, 1.2 mmol) according to the standard procedure which gave **7a** as white powder, mp = 152-154 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 2176 (C $\equiv$ N), 3325 (NH).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  : 3.42 (s, 2H,  $\text{CH}_2$ ); 3.81 (s, 3H, MeO); 4.39 (d, 2H,  $J$  = 6.4 Hz,  $\text{CH}_2\text{NH}$ ); 6.71 (dd, 1H,  $J$  = 7.6, 1.1 Hz, H-7, Ar); 6.93 (d, 1H,  $J$  = 8.1 Hz, H-5, Ar); 7.03 (t, 1H,  $J$  = 7.9 Hz, H-6, Ar); 7.23 (t, 1H,  $J$  = 7.8 Hz, H-3', Ar); 7.32 (t, 2H,  $J$  = 7.5 Hz, H-2', H-4', Ar); 7.38 (d, 2H,  $J$  = 7.0 Hz, H-1', H-5', Ar); 7.73 (t, 1H,  $J$  = 6.4 Hz, NH).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  : 24.6 ( $\text{CH}_2$ , C-4); 45.0 ( $\text{CH}_2\text{-NH}$ , C-11); 49.9 (C-3, C=); 56.4 (C-8'); 111.4 (C-7, Ar); 120.1 (CN); 121.5 (C-10, Ar); 121.5 (C-5, Ar); 124.9 (C-6, Ar); 127.4 (C-15); 128.0 (C-14, C-16, Ar); 128.3 (C-13, C-17, Ar); 138.9 (C-12, Ar); 134.3 (C-9, Ar); 147.9 (C-2, Ar); 160.4 (C-8, Ar). HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 315.1107 found (calculated for  $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2\text{Na}$   $[\text{M}+\text{Na}]^+$  requires 315.11095). Anal. Calcd for  $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_2$ : C, 73.95; H, 5.52; N, 9.58. Found: C, 73.75; H, 5.48; N, 9.56.

2.1.5.2 2-(4-Dimethylaminophenylmethyl)amino-8-methoxy-4H-chromene-3-carbonitrile (**7b**)

Compound **7b** was prepared in 96% yield (419 mg) from 2-[[*(E)*-(4-dimethylaminophenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile **6b** (434 mg, 1.2 mmol) according to the standard procedure which gave **7b** as yellowish powder, mp = 148-150 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 2173 ( $\text{C}\equiv\text{N}$ ), 3337 (NH).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.54 (s, 2H,  $\text{CH}_2$ ); 3.89 (s, 3H, MeO); 4.48 (d, 2H,  $J$  = 5.9 Hz,  $\text{CH}_2\text{NH}$ ); 4.96 (t, 1H,  $J$  = 5.3 Hz, NH); 6.77-6.63 (m, 3H, H-7, H-2', H-4', Ar); 6.81 (d, 1H,  $J$  = 8.1 Hz, H-5, Ar); 7.03 (t, 1H,  $J$  = 7.9 Hz, H-6, Ar); 7.26 (d, 2H,  $J$  = 8.5 Hz, H-1', H-5', Ar).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 24.4 ( $\text{CH}_2$ , C-4); 40.6 ( $\text{Me}_2\text{N}$ ); 45.7 (C-11); 51.1 (C-3, C=); 56.1 (C-8'); 110.4 (C-7, Ar); 112.7 (C-14, C-16, Ar); 119.9 (CN); 121.0 (C-5, Ar); 121.5 (C-10, Ar); 124.4 (C-6, Ar); 125.9 (C-12, Ar); 129.1 (C-13, C-17, Ar); 139.1 (C-9, Ar); 148.0 (C-2, Ar); 150.2 (C-8, Ar); 160.4 (C-15, Ar). HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 358.1526 found (calculated for  $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_2\text{Na}$  [ $\text{M}+\text{Na}$ ] $^+$  requires 358.1526). Anal. Calcd for  $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_2$ : C, 71.62; H, 6.31; N, 12.53. Found: C, 71.68; H, 6.36; N, 12.63.

2.1.5.3 2-(4-Methoxyphenylmethyl)amino-8-methoxy-4H-chromene-3-carbonitrile (**7c**)

Compound **7c** was prepared in 97% yield (375 mg) from 2-[[*(E)*-(4-methoxyphenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile **6c** (384 mg, 1.2 mmol) according to the standard procedure which gave **7c** as white powder, mp = 156-158 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 2172 ( $\text{C}\equiv\text{N}$ ), 3332 (NH).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.54 (s, 2H,  $\text{CH}_2$ ); 3.82 (s, 3H, MeO); 3.88 (s, 3H, MeO); 4.51 (d, 2H,  $J$  = 6.4 Hz,  $\text{CH}_2\text{NH}$ ); 5.04 (t, 1H,  $J$  = 5.6 Hz, NH); 6.69 (d, 1H,  $J$  = 7.2 Hz, H-7, Ar); 6.81 (d, 1H,  $J$  = 8.1 Hz, H-5, Ar); 6.89 (d, 2H,  $J$  = 8.6 Hz, H-2', H-4', Ar); 7.03 (t, 1H,  $J$  = 8.0 Hz, H-6, Ar); 7.31 (d, 2H,  $J$  = 8.5 Hz, H-1', H-5', Ar).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 24.4 ( $\text{CH}_2$ , C-4); 45.5 (C-11); 51.5 (C-3, C=); 55.3 ( $\text{CH}_3$ , C-15'); 56.0 ( $\text{CH}_3$ , C-8'); 110.4 (C-7, Ar); 114.1 (C-14, C-16, Ar); 119.9 (CN); 120.9 (C-5, Ar); 121.3 (C-10, Ar); 124.5 (C-6, Ar); 129.3 (C-13, C-17, Ar); 130.4 (C-12, Ar); 139.1 (C-9, Ar); 147.9 (C-2, Ar); 159.2 (C-8, Ar); 160.3 (C-15). HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 345.1206 found (calculated for  $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_3\text{Na}$  [ $\text{M}+\text{Na}$ ] $^+$  requires 345.12051). Anal. Calcd for  $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}_3$ : C, 70.79; H, 5.63; N, 8.69. Found: C, 70.81; H, 5.66; N, 8.65.

2.1.5.4 2-(4-Chlorophenylmethyl)amino-8-methoxy-4H-chromene-3-carbonitrile (**7d**)

Compound **7d** was prepared in 80% yield (314 mg) from 2-[[*(E)*-(4-chlorophenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile **6d** (389.7 mg, 1.2 mmol) according to the standard procedure which gave **7d** as yellowish powder, mp = 180-182 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 12181 ( $\text{C}\equiv\text{N}$ ), 3318 (NH).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.53 (s, 2H,  $\text{CH}_2$ ); 3.86 (s, 3H, MeO); 4.52 (d, 2H,  $J$  = 6.3 Hz,  $\text{CH}_2\text{NH}$ ); 5.16 (t, 1H,  $J$  = 5.9 Hz, NH); 6.69 (d, 1H,  $J$  = 7.4 Hz, H-7, Ar); 6.79 (d, 1H,  $J$  = 8.1 Hz, H-5, Ar); 7.03 (t, 1H,  $J$  = 8 Hz, H-6, Ar); 7.33 (s, 4H, H-1', H-5', H-2', H-4', Ar).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 24.3 ( $\text{CH}_2$ , C-4); 45.2 (C-11); 52.1 (C-3, C=); 56.0 ( $\text{CH}_3$ , C-8'); 110.4 (C-7, Ar); 119.9 (CN); 120.7 (C-5, Ar); 121.1 (C-10, Ar); 124.6 (C-6, Ar); 128.8 (C-14, C-16, Ar); 129.2 (C-13, C-17, Ar); 133.5 (C-15, Ar); 137.0 (C-12, Ar); 139.0 (C-9, Ar); 147.9 (C-2, Ar); 160.2 (C-8, Ar); HRMS ( $\text{ES}^+$ ,  $\text{MeOH}/\text{CH}_2\text{Cl}_2$  9:1)  $m/z$ : 349.0714 found [ $\text{M}+\text{Na}$ ] $^+$  (calculated for  $\text{C}_{18}\text{H}_{15}\text{N}_2\text{O}_2^{35}\text{ClNa}$  [ $\text{M}+\text{Na}$ ] $^+$  requires 349.07198). Anal. Calcd for  $\text{C}_{18}\text{H}_{15}\text{N}_2\text{O}_2\text{Cl}$ : C, 66.16; H, 4.63; N, 8.57. Found: C, 66.12; H, 4.62; N, 8.59.

2.1.5.5 2-(3-Methoxyphenylmethyl)amino-8-methoxy-4H-chromene-3-carbonitrile (**7e**)

Compound **7e** was prepared in 78% yield (302 mg) from 2-[[*(E)*-(3-methoxyphenyl)methylene]amino]-8-methoxy-4H-chromene-3-carbonitrile **6e** (384 mg, 1.2 mmol) according to the standard procedure which gave **7e** as white powder, mp = 144-146 °C. IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 2176 ( $\text{C}\equiv\text{N}$ ), 3362 (NH).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 3.45 (s, 2H,  $\text{CH}_2$ ); 3.72 (s, 3H, MeO); 3.77 (s, 3H, MeO); 4.45 (d, 2H,  $J$  = 6.2 Hz,  $\text{CH}_2\text{NH}$ ); 4.99 (t, 1H,  $J$  = 5.8 Hz, NH); 6.60 (d, 1H,  $J$  = 7.7 Hz, H-7, Ar); 6.70 (d, 1H,  $J$  = 8.2 Hz, H-5, Ar); 6.76 (dd, 1H,  $J$  = 8.3, 2.4 Hz, H-3', Ar); 6.87 (d, 2H,  $J$  = 8.1 Hz, H-1', H-5', Ar); 6.94 (t, 1H,  $J$  = 8 Hz, H-6, Ar); 7.19 (t, 1H,  $J$  = 7.8 Hz, H-4', Ar).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  : 24.3 ( $\text{CH}_2$ , C-4); 45.9 (C-11); 51.8 (C-3, C=); 55.2 ( $\text{CH}_3$ , C-14'); 56.0 ( $\text{CH}_3$ , C-8'); 110.4 (C-7, Ar); 113.2 (C-15, Ar); 113.4 (C-13, Ar); 119.9 (CN); 120.1 (C-5, Ar); 120.9 (C-17, Ar); 121.2 (C-10, Ar); 124.5 (C-6,



Ar); 129.8 (C-16, Ar); 139.1 (C-9, Ar); 139.9 (C-12, Ar); 147.9 (C-2, Ar); 159.9 (C-8, Ar); 160.3 (C-14, Ar). HRMS ( $ES^+$ , MeOH/CH<sub>2</sub>Cl<sub>2</sub> 9:1)  $m/z$ : 345.1214 found (calculated for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>Na [M+Na]<sup>+</sup> requires 345.12151). Anal. Calcd for C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.80; H, 5.62; N, 8.70.

## 2.2 Protein kinase assays

Kinase activities were assayed in appropriate kinase buffer, with either protein or peptide as substrate in the presence of 15  $\mu$ M [ $\gamma$ -<sup>33</sup>P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30  $\mu$ l following the assay described in literature<sup>14</sup>. Controls were performed with appropriate dilutions of dimethylsulfoxide. Full-length kinases are used unless specified. Peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

- CDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer A, with 0.8  $\mu$ g/ $\mu$ l of histone H1 as substrate.
- CDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer B (+ 0.15 mg/ml of BSA + 0.23 mg/ml of DTT) with 0.27  $\mu$ g/ $\mu$ l of the following peptide: YSPTSPSYSPSYSPSYSPSKKKK, as substrate.
- PIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer A with 0.8  $\mu$ g/ $\mu$ l of histone H1 (Sigma #H5505) as substrate.
- Haspin (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer C with 0.007  $\mu$ g/ $\mu$ l of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate;
- SscGSK-3 $\alpha$ / $\beta$  (glycogen synthase kinase-3 $\alpha$ / $\beta$ , native, affinity purified from porcine brain extracts, *Sus scrofa domestica*) was assayed in buffer B (+ 0.15 mg/ml of BSA + 0.23 mg/ml of DTT), with 0.010  $\mu$ g/ $\mu$ l of GS-1 peptide, a GSK-3-selective substrate (YRRAVPPSPSLSRHSSPHQSpEDEEE, "Sp" stands for phosphorylated serine).
- SscCK1 $\delta$ / $\epsilon$  (porcin casein kinase 1 $\delta$ / $\epsilon$ , native, affinity purified from porcine brain extracts, *Sus scrofa domestica*) was assayed in buffer A, with 0.022  $\mu$ g/ $\mu$ l of the following peptide: RRKHAAGSpAYSITA as CK1-specific substrate.

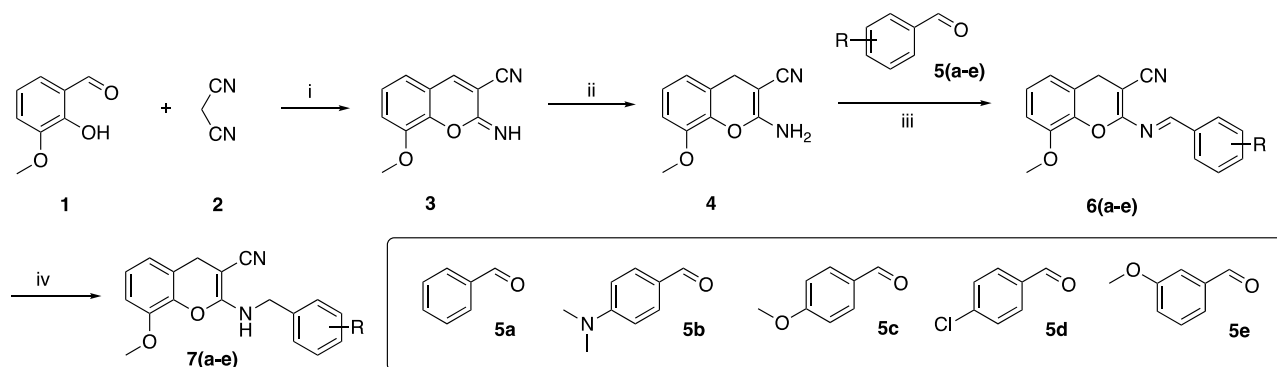
### Buffers:

- (A) 60 mM  $\beta$ -glycerophosphate, 30 mM *p*-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM MgCl<sub>2</sub>, 1 mM DTT, 0.1 mM sodium orthovanadate;
- (B) 10 mM MgCl<sub>2</sub>, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50  $\mu$ g/ml heparin;
- (C) MOPS 25 mM pH 7.5, 10 mM MgCl<sub>2</sub>.

## 3 Results and discussion

### 3.1 Chemistry and characterizations of synthesized compounds

Access to the planned 2-benzylamino-4*H*-chromene-3-carbonitrile **7** from the 2-amino-4*H*-chromene-3-carbonitrile platform **4** is outlined in Scheme 1.



**Scheme 1:** Synthetic route of the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)**. *Reagent and conditions:* i) piperidine 0.5% mol., EtOH, 25°C, 4 h. ii) NaBH<sub>4</sub>, MeOH, 25°C, 20 min. iii) piperidine, EtOH, 80°C, 5 h. iv) NaBH<sub>4</sub>, THF, 0°C, 20 min.

The preparation of key intermediate **4** involved a two-step approach developed in our laboratory<sup>11</sup>. The first step is based on a facile hetero cyclization between 2-hydroxy-3-methoxybenzaldehyde or *o*-vanillin **1** and propanedinitrile **2** catalyzed with piperidine 0.5% mol. in ethanolic solution at room temperature, which afforded the 8-methoxy-2-imino-2*H*-[1]-benzopyran-3-carbonitrile **3** in 85% yield after work-up. In the second step, the imino-coumarin **3** was transformed into the 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile **4** (in 75% yield) by reduction in methanolic solution of sodium borohydride. With the platform **4** in hand, initial attempts to obtain the desired *N*-benzylamino-4*H*-chromene-3-carbonitrile by direct *N*-alkylation of platform **4** with various benzyl halide were unsuccessful. After a set of experiments for the preparation of mono alkylated derivatives **7** in varying the following parameters as - reaction time range (6-24 hours), - the ratio of reagents (**4** / benzyl halide: 1.05-1.1), - reaction temperature, - use of non-polar or polar solvents, we observed many difficulties to control mono alkylation or, to have a minimum of reactivity of the halide reagents.

Therefore, we decided to abandon this *N*-alkylation approach and to explore the reductive amination. After optimization of the reaction conditions for transformation of platform **4** into the corresponding aldimine **6a** with benzaldehyde **5a** in the presence of 1 equivalent of piperidine in refluxed absolute ethanol (reaction time: 5 h, **6a**: 70% yield), we extended this reaction with various aldehydes **5(b-c)** in order to introduce molecular diversity. All the aldimines **6(a-e)** were isolated from the reaction mixture by cooling, the insoluble compounds **6** were collected by a simple filtration and rinsed with cooled absolute ethanol. The desired compounds **6(a-e)** were synthesized in yields ranging from 52 to 90% (Table 1). Structure of compounds **6(a-e)** was substantiated by FTIR (CN stretch, 2196 <  $\nu$  (cm<sup>-1</sup>) < 2206), by <sup>1</sup>H NMR (N=CH signal, 8.81 <  $\delta$  < 8.92 ppm which is in agreement with a *E*-configuration<sup>15</sup> and <sup>13</sup>C NMR (=CH,  $\delta$  160.50 ppm). In mass spectrometry analysis (HRMS) the [M+Na]<sup>+</sup> molecular ion signal for the five compounds **6** were readily obtained as base signal.

**Table 1:** Results for the preparation of 2-[(*E*)-(benzylidene)amino]-8-methoxy-4*H*-chromene-3-carbonitrile **6(a-e)** and 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)**.

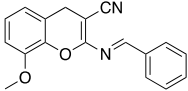
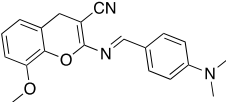
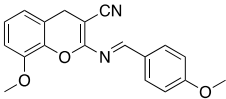
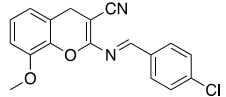
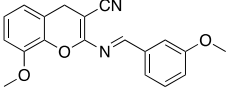
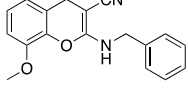
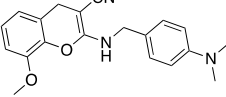
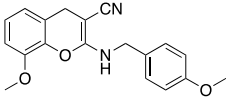
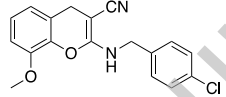
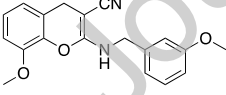
Structure of compound <b>6</b> and <b>7</b>	Yield (%) <sup>a</sup>	Overall yield (%) <sup>b</sup>	$\delta_{\text{CH=}}$ of <b>6</b> (ppm) <sup>c</sup>	$\delta_{\text{CH}_2\text{Ar}}$ of <b>7</b> (ppm) <sup>e</sup>	$\nu_{\text{NH}}$ of <b>7</b> (cm <sup>-1</sup> )
<b>6a</b>	70	45	8.88 <sup>d</sup>	162.4 <sup>d</sup>	-

<b>6b</b>		52	33	8.81	160.8	-	-
<b>6c</b>		90	57	8.88	160.9	-	-
<b>6d</b>		80	51	8.89	160.2	-	-
<b>6e</b>		78	50	8.92	161.7	-	-
<b>7a</b>		96	43	-	-	4.38 <sup>d</sup>	3325
<b>7b</b>		96	32	-	-	4.48	3337
<b>7c</b>		97	56	-	-	4.51	3332
<b>7d</b>		80	41	-	-	4.51	3318
<b>7e</b>		78	39	-	-	4.45	3362

<sup>a</sup> Yield of isolated product **6** and **7**. <sup>b</sup> Overall yield of **6** and **7** calculated from compound **3**. <sup>c</sup> <sup>1</sup>H NMR and <sup>13</sup>C NMR in CDCl<sub>3</sub> solution. <sup>d</sup> <sup>1</sup>H NMR and <sup>13</sup>C NMR in DMSO-*d*<sub>6</sub> solution with TMS as internal reference. <sup>e</sup> <sup>1</sup>H NMR in CDCl<sub>3</sub> solution.

Finally, treatment of aldimines **6(a-e)** with sodium borohydride in cooled THF solution (0°C) during 20 min., afforded the reduced compounds **7(a-e)**. After elimination of the solvent *in vacuo*, the crude residue was triturated with deionized water and insoluble material was collected by filtration, and eventually recrystallized from absolute ethanol. Inspection of the data presented in Table 1 showed that reduction works well (**7(a-e)**: 78-96% yield) and the targeted 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** were prepared in good overall yields (32-56%). Before entering the biological tests, the five products **7** were characterized by <sup>1</sup>H, <sup>13</sup>C NMR, FTIR, HRMS and centesimal analysis. Examination of their <sup>1</sup>H NMR spectra in DMSO-*d*<sub>6</sub> solution showed that the CH<sub>2</sub>Ar signal is located in the range 4.38 < δ < 4.51 ppm and in the IR spectrum, important bands of absorption were observed between 3318 and 3361 cm<sup>-1</sup> which can be attributed to the N-H stretching frequencies (Table 1).

3.2 Physicochemical properties and *in silico* ADME studies**Table 2:** Results of physicochemical properties for compounds **6(a-e)** and **7(a-e)**.

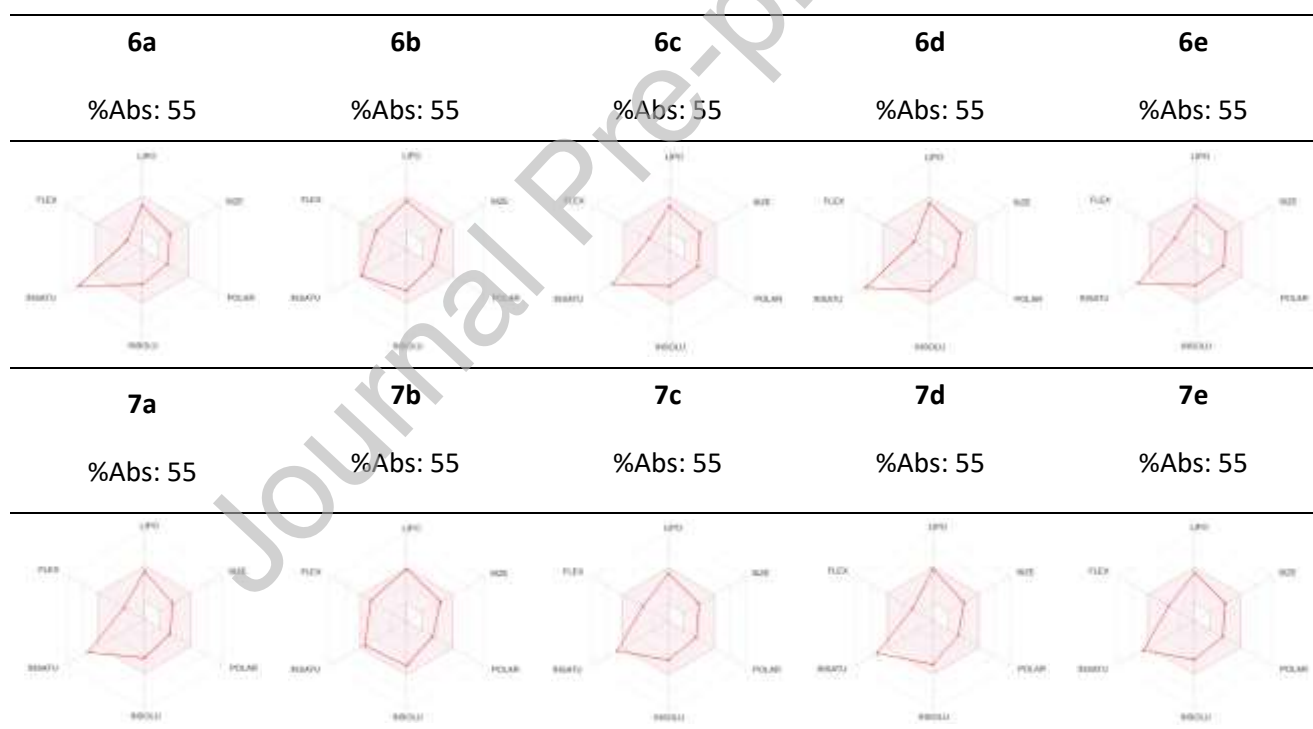
Prod.	Structure	MW (g/mol)	Log P	Log S	tPSA (Å <sup>2</sup> )	HBA	HBD	RB	Fract. Csp <sup>3</sup>	LV
<b>6a</b>		290.32	3.26	-3.89	54.61	4	0	3	0.11	0
<b>6b</b>		361.44	3.95	-4.60	57.85	4	0	6	0.27	0
<b>6c</b>		320.34	3.27	-3.95	63.84	5	0	4	0.16	0
<b>6d</b>		324.76	3.80	-4.48	54.61	4	0	3	0.11	0
<b>6e</b>		320.34	3.27	-3.95	63.84	5	0	4	0.16	0
<b>7a</b>		293.33	3.13	-4.29	54.28	3	1	4	0.17	0
<b>7b</b>		363.45	3.75	-5.00	57.62	3	1	7	0.32	0
<b>7c</b>		322.36	3.10	-4.36	63.51	4	1	5	0.21	0
<b>7d</b>		326.78	3.67	-4.89	54.28	3	1	4	0.17	0
<b>7e</b>		322.36	3.13	-4.36	63.51	4	1	5	0.21	0

MW, molecular weight; Log P, partition coefficient; Log S, water solubility; tPSA, topological surface area; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; RB, rotatable bonds, LV, number of Lipinski's rule of 5 violations.

The physicochemical properties of compounds **6(a-e)** and **7(a-e)** were calculated with the Swiss ADME<sup>16</sup> server platform<sup>17</sup>. These physicochemical properties were evaluated based on Lipinski's rules<sup>18</sup>, considering log P (octanol/water partition coefficient) which indicates the concentration ratio of a neutral substance between two immiscible solvents in equilibrium and reveals the differential solubility of this substance in the two solvents. Log P is a very important parameter because it influences the bioavailability, permeability and toxicity of a drug candidate and a drug. Molecular weight (MW), aqueous solubility (log S), topological polar surface area (tPSA), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA)<sup>19</sup>, number of rotatable bonds (RB), the fraction of Csp<sup>3</sup> and drug-likeness (violation of the 5 Lipinski rules) completed this *in silico* analysis (Table 2).

The log P values of compounds **6(a-e)** and **7(a-e)** were acceptable, all under 5.0 and none of them negative. The highest values (3.75 and 3.80) correspond to **7b** ( $R = p\text{-NEt}_2$ ) and to **6d** ( $R = p\text{-Cl}$ ). According to the calculated values of aqueous solubility (log S), most of the aldimines **6(a-e)** and the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** are moderately soluble in water ( $-4 < \log S_{6(a-e)} < -3.9$  and  $-5 < \log S_{7(a-e)} < -4.3$ ). For predicting the drug solubility, there is another effective descriptor for transport properties of a molecule is the topological surface area (tPSA) which described the sum of the surfaces of its polar atoms (N, O and Cl), and the hydrogen atoms attached to them. tPSA is an interacting indicator of gastrointestinal absorption and penetration of the brain-blood barrier (BBB). For compounds **6(a-e)** and **7(a-e)**, tPSA values from 54 to 64 Å<sup>2</sup> evidenced an acceptable permeability. Complete analysis of the data in Table 2 shows that the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** and their corresponding precursors **6(a-e)** are in agreement with Lipinski's rules of five (R05)<sup>20</sup> for the following requirements: (a) a molecular weight < 500 g/mole; (b) hydrogen bond donors HBD < 5; (c) hydrogen bond acceptors HBA < 10<sup>21</sup>; (d) Log P < 4.15 and (e) tPSA ≤ 140 Å<sup>2</sup>. This means that these compounds should have efficient absorption and good oral potential bioavailability<sup>22</sup>.

**Table 3:** Bioavailability radar related to the physicochemical properties of compounds **6(a-e)** and **7(a-e)**. The pink colored zone is the suitable physicochemical space for oral bioavailability. LIPO (Lipophilicity):  $-0.7 < \log P < +5.0$ ; SIZE:  $150 \text{ g/mol} < \text{MW} < 500 \text{ g/mol}$ ; POLAR (Polarity):  $20 \text{ Å}^2 < \text{tPSA} < 130 \text{ Å}^2$ ; INSOLU (Insolubility):  $-6 < \log S < 0$ ; INSATU (Insaturation):  $0.25 < \text{Fraction Csp}^3 < 1$ ; FLEX (Flexibility):  $0 < \text{Number rotatable bonds} < 9$ .

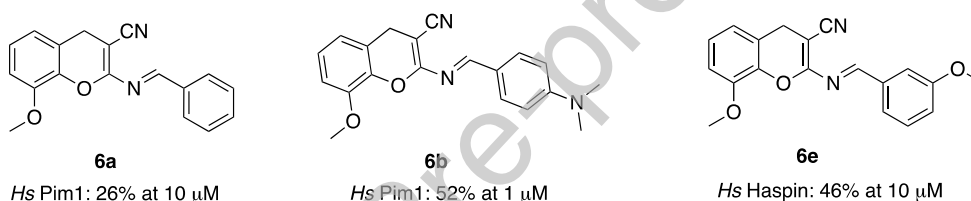


Using the SwissADME server<sup>17</sup>, the scale of similarity of synthetic compounds with the drug was used to evaluate criteria integrating lipophilicity (LIPO), size (MW), polarity (tPSA), solubility (log S), flexibility (FLEX, see also the number of rotatable bonds RB in Table 2), and saturation (INSATU). The ideal situation is that when all properties of these molecules are located in the pink region. That is to say when the basic constant properties are on the radar chart. The biocompatibility radar graphs and bioavailability of compounds **6(a-e)** and **7(a-e)** are reported in Table 3. Except for saturation, the radar plot shows whether all compounds **6** and **7** are in the pink area. It should be noted that the bioavailability score % (%Abs) of all compounds is 55%.

### 3.3 *In vitro* protein kinases assays

Now for the 2<sup>nd</sup> part of this study related to the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** and their aldimine precursors **6(a-e)**, we have been interested in exploring their biological properties, particularly on six different *in vitro* kinase assays. The protein kinases selected for these *in vitro* assays are the following: HsCDK5-p25 (*Homo sapiens* cyclin-dependant kinase 5-p25) <sup>23</sup>; HsCDK9/cyclin T (*Homo sapiens* cyclin-dependant kinase 9) <sup>24</sup>; HsPim1 (*Homo sapiens* Pim1, proto-oncogene, serine/threonine kinase) <sup>25</sup>; HsHaspin (*Homo sapiens* Haspin, a mitotic serine/threonine protein kinase); SscGSK-3 $\alpha/\beta$  (*Sus scrofa domestica* glycogene synthase kinase-3 $\alpha/\beta$ ) <sup>26</sup> and SscCK1 $\delta/\epsilon$  (*Sus scrofa domestica* casein kinase1 $\delta/\epsilon$ ) <sup>27</sup>. All the selected enzymes presented interest for protein phosphorylation of serine, threonine and tyrosine residues in many regulatory mechanisms of cells. Dereglulation of these protein kinases is mainly responsible of human diseases <sup>28</sup>.

In Table 4 are reported the results of *in vitro* kinase assays with the rate (%) of residual activities at 10  $\mu$ M and 1  $\mu$ M. Analysis of these results showed three categories of compounds. The first category is related to compounds which presented no significant protein kinase activity (rate > 60-70% at 10  $\mu$ M and 1  $\mu$ M). The second category integrated compounds with moderate interest and the rate is less than or close to 50%. For the third category, the rate is less than 50% at 10  $\mu$ M and at 1  $\mu$ M, so the tested compounds have a marked effect on the catalytic activity.



**Figure 3:** 2-Benzylidenamino-8-methoxy-4*H*-chromene-3-carbonitrile **6(a,b)** and **6e** which are bioactive against protein kinases Pim1 and Haspin. *Hs*, *Homo sapiens*.

Results given in Table 4 showed that none compound **7** presents inhibition effects on this panel of these six protein kinases, but this does not mean that compounds **7** are inactive against protein kinases, it concerns only this panel. However, and among the 2-benzylidenamino-8-methoxy-4*H*-chromene-3-carbonitrile **6(a-e)**, three compounds exhibited inhibitory activity on Pim1 and Haspin (Figure 3). For Pim1, this concerns the compound **6a** (26% rate at 10  $\mu$ M) and **6b** (55% rate at 1 mM); for Haspin, only the compound **6e** is active (46% rate at 10  $\mu$ M).

**Table 4:** Effects of the 2-benzylidenamino-8-methoxy-4*H*-chromene-3-carbonitrile **6(a-e)** and the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)** on the catalytic activity of six purified protein kinases. ATP concentration used in the kinase assays was 15  $\mu$ mol/L. Results are expressed in % of maximal activity, *i.e.* measured in the absence of inhibitor but with an equivalent dose of DMSO (solvent of the tested compounds) (values are means, *n* = 2). Kinases are from human origin except when it is specified *Ssc*, *Sus scrofa domestica*.

Compound	Concentration ( $\mu$ M)	CDK5-p25	CDK9/cyclin T	Pim1	Haspin *	SscGSK 3 $\alpha$ / $\beta$	SscCK1 $\delta$ / $\epsilon$
<b>6a</b>	10	$\geq 100$	77	26	79	83	63
	1	$\geq 100$	104	99	98	77	$\geq 100$
<b>6b</b>	10	$\geq 100$	88	55	71	92	49
	1	99	93	52	80	89	75
<b>6c</b>	10	94	$\geq 100$	$\geq 100$	86	88	$\geq 100$
	1	100	$\geq 100$	$\geq 100$	96	83	69
<b>6d</b>	10	97	79	60	68	65	72
	1	95	72	$\geq 100$	$\geq 100$	80	59
<b>6e</b>	10	$\geq 100$	88	73	46	71	$\geq 100$
	1	$\geq 100$	83	88	69	73	$\geq 100$
<b>7a</b>	10	84	$\geq 100$	81	$\geq 100$	$\geq 100$	97
	1	$\geq 100$	96	86	$\geq 100$	100	97
<b>7b</b>	10	$\geq 100$	85	100	$\geq 100$	93	$\geq 100$
	1	$\geq 100$	$\geq 100$	87	97	99	$\geq 100$
<b>7c</b>	10	$\geq 100$	88	73	99	84	$\geq 100$
	1	$\geq 100$	84	74	66	97	92
<b>7d</b>	10	97	89	68	71	91	86
	1	$\geq 100$	88	$\geq 100$	$\geq 100$	$\geq 100$	74
<b>7e</b>	10	$\geq 100$	79	87	$\geq 100$	75	59
	1	$\geq 100$	84	76	59	75	83

NB:  $\geq 100$  indicates that the compound cannot inhibit the enzymatic activity at the tested concentration. \* For Haspin, the kinase domain (amino acids 470 to 798) was expressed and analyzed.

#### 4 Conclusion

For this study, we have described a practical and new approach to 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7** with good overall yields ranging from 32 to 56%. The key step of this methodology is the reductive amination from the platform **4** which afforded indirectly an access to mono substitution of the amino function in position C-2 for this platform. The physicochemical properties (log P,

log S, tPSA, HBA, HBD, fraction Csp<sup>3</sup>, number of rotatable bond) have been established and results are in agreement with Lipinski's rules of five (R05). The ADME properties of the compounds **6(a-e)** and **7(a-e)** were also extensively studied *in silico*. Exploration of biological activities was realized against a panel of six protein kinases and among the 8-methoxy-4*H*-chromene-3-carbonitrile **6** and **7**, only compounds **6(a,b)** and **6e** are potentially interesting because they showed good percentage of residual activities against Pim1 and Haspin. The current results are the beginning of a next relationship structure-activity (RSA) program for identification of a better promising protein kinase inhibitor derived from the 2-amino-4*H*-chromene-3-carbonitrile platform. Works are in progress in our laboratory.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationships that have appeared to influence the work reported in this paper.

### Acknowledgments

One of us (M.D.) wishes to thank the "Ministère de l'Enseignement Supérieur et de la Recherche de Tunisie" for the grant. Financial support of this program carried out under the French National Cancer Institute "Cancéropole Grand Ouest" in the "Molécules Marines, Métabolisme et Cancer" M3C network, is gratefully acknowledged. S. B. and T. R. thank IBiSA (French Infrastructures en Sciences du Vivant: Biologie, Santé et Agronomie) and Biogenouest (Western France life science and environment core facility network supported by the Conseil Régional de Bretagne) for supporting the *KISSf* screening facility (FR2424, CNRS and Sorbonne Université). The authors are grateful to the assistance of the staff of the CRMPO analytical chemistry core facility for HRMS analysis (CRMPO platform, ScanMAT UAR 2025 CNRS at University of Rennes 1, Building 11A, Campus de Beaulieu, Rennes, France).

### Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molstruc.2022.yyyyyy>.

### References

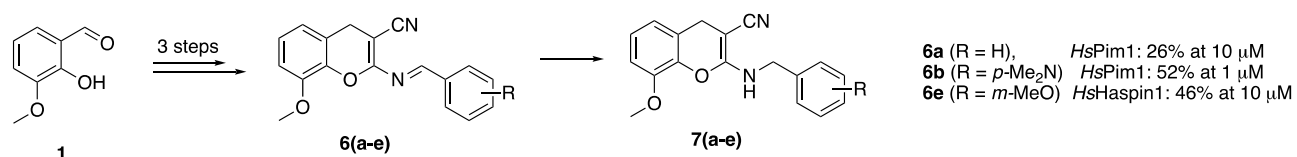
- [1] S.A. Patil, R. Patil, L.M. Pfeffer, D.D. Miller. Chromenes: potential new chemotherapeutic agents for cancer. *Future Med Chem* 5 (2013) 1647-60. <https://doi.org/10.4155/fmc.13.126>
- [2] a) S.X. Cai, J.A. Drewe, S. Kasibhatla, W.E. Kemnitzer, B.Y. Tseng, C. Blais, H. Gourdeau, D. Labrecque. Substituted 4-aryl chromene as activator of caspases and inducer of apoptosis and as anti-vascular agent and the use thereof. Patent WO2008/005572 A2, 6 July 2006.  
b) W. Kemnitzer, K. Shailaja, J. Songchun, Z. Zhang, J. Zhao, S. Jia, L. Xu, C. Crogan-Grundy, R. Denis, N. Barriault, L. Vaillancourt, S. Charron, J. Dodd, G. Attardo, D. Labrecque, S. Lamothe, H. Gourdeau, B. Tseng, J. Drewe, S.X. Cai. Discovery of 4-aryl-4*H*-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. 2. Structure-activity relationships of the 7- and 5-, 6-, 8-positions. *Bioorg Med Chem Letters* 15 (2005) 4745-4751. <https://doi.org/10.1016/j.bmcl.2005.07.066>
- [3] J.M.D. Gulley. (2010) A Phase I/II trial of crolibulin (EPC2407) plus cisplatin in adults with solid tumors with a focus on anaplastic thyroid cancer (ATC). Accessed 21 July 2022. <http://www.clinicaltrials.gov>
- [4] S. Kasibhatla, H. Gourdeau, K. Meerovitch, J. Drewe, S. Reddy, L. Qiu, H. Zhang, F. Bergeron, D. Bouffard, Q. Yang, J. Herich, S. Lamothe, S.X. Cai, B. Tseng. Discovery and mechanism of action of a novel series of apoptosis inducers with potential vascular targeting activity. *Mol Cancer Ther* 11 (2004) 1365-1374. <https://doi.org/10.1158/1535-7163.1365.3.11>



- [5] H. Gourdeau, L. Leblond, B. Hamelin, C. Desputeau, K. Dong, I. Kianicka, D. Custeau, C. Boudreau, L. Geerts, S.X. Cai, J. Drewe, D. Labrecque, S. Kasibhatla, B. Tseng. Anti-vascular and antitumor evaluation of 2-amino-4-(bromo-4,5-dimethoxyphenyl)-3-cyano-4*H*-chromenes, a novel series of anticancer agents. *Mol Cancer Ther* 11 (2004) 1375-1384. <https://doi.org/10.1158/1535-7163.1375.3.11>
- [6] F. Manero, F. Gautier, T. Gallenne, N. Cauquil, D. Grée, P-F. Cartron, O. Geneste, R. Grée, F.M. Vallette, P. Juin. The small organic compound HA14-1 prevents Bcl-2 interaction with Bax to sensitize malignant glioma cells to induction of cell death. *Cancer Res.* 66 (2006) 2757-2764. <https://doi.org/10.1158/0008-5472.CAN-05-2097>
- [7] J.M. Doshi, D. Tian, C. Xing. Structure activity relationship studies of ethyl-2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (HA 14-1), an antagonist for antiapoptotic Bcl-2 proteins to overcome drug resistance in cancer. *ACS J Med Chem* 49 (2006) 7731-7739. <https://doi.org/10.1021/jm060968r>
- [8] M. Weyland, F. Manero, A. Paillard, D. Grée, G. Vialt, D. Jarnet, P. Menei, P. Juin, I. Chourpa, J-P. Benoit, R. Grée, E. Garcion. Mitochondrial targeting by use of lipid nanocapsules loaded with SV30, an analogue of the small-molecule Bcl-2 inhibitor HA 14-1. *J Control Release* 151 (2011) 74-82. <https://doi.org/10.1016/j.jconrel.2010.11.032>
- [9] D.L. Wood, D. Panda, T.R. Wiernicki, L. Wilson, M.A. Jordan, J.P. Singh. Inhibition of mitosis and microtubule function through direct tubulin binding by a novel antiproliferative naphthopyran LY290181. *Mol Pharm* 52 (1997) 437-444. <https://doi.org/10.1124/mol.52.3.437>
- [10] D. Panda, J.P. Singh, L. Wilson. Suppression of microtubule dynamics by LY290181. *J Biol Chem* 272 (1997) 7681-7687. <https://doi.org/10.1074/jbc.272.12.7681>
- [11] A. Bouattour, M. Fakhfakh, S. Abid, L. Paquin, R. Le Guével, A. Corlu, S. Ruchaud, S. Bach, H. Ammar, J-P. Bazureau. Microwave assisted a practical synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives and exploration of their biological activities. *Arkivoc* 4 (2017) 291-302. <https://doi.org/10.24820/ark.5550190.p010.040>
- [12] A. Bouattour, M. Fakhfakh, S. Abid, L. Paquin, H. Ammar, J-P. Bazureau. An efficient and concise syntheses of *N*-3-substituted 9-methoxy-4*H*-[1]-benzopyrano[2,3-*d*]pyrimidine-4(5*H*)-imine and formamidine derivatives from methyl *N*-(3-cyano-8-methoxy-4*H*-[1]-benzopyran-2-yl)methanimidate. *Synthesis* 49 (2017) 3768-3774. <https://doi.org/10.1055/s-0036-1588825>
- [13] A. Bouattour, M. Fakhfakh, S. Abid, L. Paquin, R. Le Guével, T. Charlier, S. Ruchaud, S. Bach, J-P. Bazureau, H. Ammar. Synthesis of new 2-phenylamino-4*H*-chromene-3-carbonitrile derivatives and their effects on tumor cell lines and against protein kinases. *Int J Org Chem* 10 (2020) 88-103. <https://doi.org/10.4236/ijoc.2020.102006>
- [14] S. Bach, M. Knockaert, J. Reinhardt, O. Lozach, S. Schmitt, B. Baratte, M. Koken, S.P. Coburn, L. Tang, T. Jiang, D.C. Liang, H. Galons, J-F. Dierick, L.A. Pinna, F. Meggio, F. Totzke, C. Schächtele, A.S. Lerman, A. Carnero, Y. Wan, N. Gray, L. Meijer. Roscovitine targets, protein kinases and pyridoxal kinase. *J Biol Chem* 280 (2005) 31208-31219. doi: 10.1074/jbc.M500806200.
- [15] J. Bjørge, D.R. Boyd, C. Watson, W.B. Jennings, D.M. Jerina. E-Z -Isomerism in aldimines. *J Chem Soc Perkin II* (1974) 1081-1084. doi: [10.1039/P29740001081](https://doi.org/10.1039/P29740001081)
- [16] A. Daina, O. Michielin, V. Zoete. SwissADME: a free web tool to. evaluate pharmacokinetics, druglikeness and medicinal chemistry friendliness of small molecules. *Nature Sc. Rep* 7 (2017) 42717. doi: 10.1038/srep42717
- [17] Swiss ADME of Swiss Institute of Bioinformatics. Accessed 4 May 2022. <http://www.swissadme.ch/>

- [18] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46 (2001) 3-26. doi:[10.1016/S0169-409X\(00\)00129-0](https://doi.org/10.1016/S0169-409X(00)00129-0)
- [19] C.A. Lipinski. Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol* 1 (2004) 337-41. doi: 10.1016/j.ddtec.2004.11.007
- [20] C. Abad-Zapatero. A sorcerer's apprentice and the rule of five: from rule-of-thumb to commandment and beyond. *Drug Discov. Today* 12 (2007) 995-997. doi: [10.1016/j.drudis.2007.10.022](https://doi.org/10.1016/j.drudis.2007.10.022)
- [21] A. Alex, D.S. Millan, M. Perez, F. Wakenhut, G.A. Whitlock. Intramolecular hydrogen bonding to improve membrane permeability and absorption in beyond rule of five chemical space. *MedChemComm* 2 (2011) 669-674. <https://doi.org/10.1039/c1md00093d>
- [22] B.C. Doak, B. Over, F. Giordanetto, J. Kihlberg. Oral druggable space beyond the rule of 5: Insights from drugs and clinical candidates. *Chem Biol* 21 (2014) 1115-1142. <http://dx.doi.org/10.1016/j.chembiol.2014.08.013>
- [23] S. Leclerc, M. Garnier, R. Hoessel, D. Marko, J.A. Bibb, G.L. Snyder, P. Greengard, J. Biernat, E.M. Mandelkow, G. Eisenbrand, L. Meijer. Indirubins inhibit glycogen synthase kinase-3 $\beta$  and CDK5/P25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin dependent kinase inhibitors? *J Biol Chem* 276 (2001) 251-260. <https://doi.org/10.1074/jbc.M002466200>
- [24] Y.A. Sonawane, M.A. Taylor, J.V. Napoleon, S. Rana, J.I. Contreras, A. Natarajan. Cyclin dependent kinase 9 inhibitors for cancer therapy. *ACS J Med Chem* 59 (2016) 8667-8684. <https://doi.org/10.1021/acs.jmedchem.6b00150>
- [25] C. Blanco-Aparicio, A. Carnero. Pim kinases in cancer: Diagnostic, prognostic and treatment opportunities. *Biochem Pharm* 85 (2013) 629-643. <https://doi.org/10.1016/j.bcp.2012.09.018>
- [26] A. Primot, B. Baratte, M. Gompel, A. Borgne, S. Liabeuf, J.L. Romette, F. Costantini, L. Meijer. Purification of GSK-3 by affinity chromatography on immobilized axin. *Protein Expr Purif* 20 (2000) 394-404. <https://doi.org/10.1006/prep.2000.1321>
- [27] G. Rena, J. Bain, M. Elliott, P. Cohen. D4476, a cell-permeant inhibitor of CK1, suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. *EMBO Reports* 5 (2004) 60-65. <https://doi.org/10.1038/sj.embor.7400048>
- [28] M. Buljan, R. Ciuffa, A. van Drogen, A. Vichalkovski, M. Mehnert, G. Rosenberger, S. Lee, M. Varjosalo, L.E. Pernas, V. Spegg, B. Snijder, R. Aebersold, M. Gstaiger. Kinase interaction network expands functional and disease roles of human kinases. *Molecular Cell* 79 (2020) 504-520. <https://doi.org/10.1016/j.molcel.2020.07.001>

## Graphical abstract






Journal Pre-proof

**Highlights**

- 2-Benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile
- Reductive amination
- Lipinski's descriptors
- Protein kinase
- *HsPim1* inhibitor

Journal Pre-proof

# Practical approach to *N*-benzyl derivatives of 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile by reductive amination: exploration of their effects against protein kinases and *in silico* ADME profiling

Marwa Dhiabi <sup>a,b</sup>, Ali Bouattour <sup>a</sup>, Mehdi Fakhfakh <sup>a</sup>, Souhir Abid <sup>c</sup>, Ludovic Paquin <sup>b, </sup>, Thomas Robert <sup>d,e</sup>, Stephane Bach <sup>d,e, </sup>, Jean-Pierre Bazureau <sup>b,\*, </sup> and Houcine Ammar <sup>a</sup>

<sup>a</sup> *Laboratoire de Chimie Appliquée: Hétérocycles, Corps Gras et Polymères. Faculté des Sciences de Sfax. Université de Sfax, Route Soukra, BP 1171, 3000 Sfax, Tunisie.*

<sup>b</sup> *Institut des Sciences Chimiques de Rennes ISCR UMR CNRS 6226, Université de Rennes 1, Bât. 10A, Campus de Beaulieu, CS 74205, 263 Avenue du Général Leclerc, 35042 Rennes Cedex, France.*

<sup>c</sup> *Al-Jouf University, Department of Chemistry (Science and Arts): Al Qurayat, Al Jawf, Saudi Arabia.*

<sup>d</sup> *Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models Laboratory (LBI2M), Station Biologique de Roscoff, Place George Tessier, BP 74, 29680 Roscoff, France.*

<sup>e</sup> *Sorbonne Université, CNRS, FR2424, Plateforme de criblage KISSf (Kinase Inhibitor Specialized Screening facility), Station Biologique de Roscoff, Place George Plessier, BP 74, 29680 Roscoff, France.*

Corresponding author : [jean-pierre.bazureau@univ-rennes1.fr](mailto:jean-pierre.bazureau@univ-rennes1.fr), phone: +33 223 236 603

## Declaration of interests

- ☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- ☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof