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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

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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-3a/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

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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 [1]. For examples in Figure 1, EPC 2407 (**I**) or crolibulin™ was identified in clinical phase I/II trials as drug-candidate [2] for the treatment of advanced solid tumors on anaplastic thyroid cancer (ATC). During the randomized phase II trial (NTC 01240590) [3], 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**) [4] was evaluated as apoptosis inducer (EC_{50} 50 nM) and inhibited cell growth of TD47D breast cancer cells [5] 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 [6] using a *de novo* computer-aided design strategy based on the predicted structure of Bcl-2 protein [7]. 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 [8]. For the naphtopyran derivative LY 290161 (**V**) [9], it showed antiproliferative activity on microtubulin binding, particularly in the G2/M phase of the cell cycle [10].

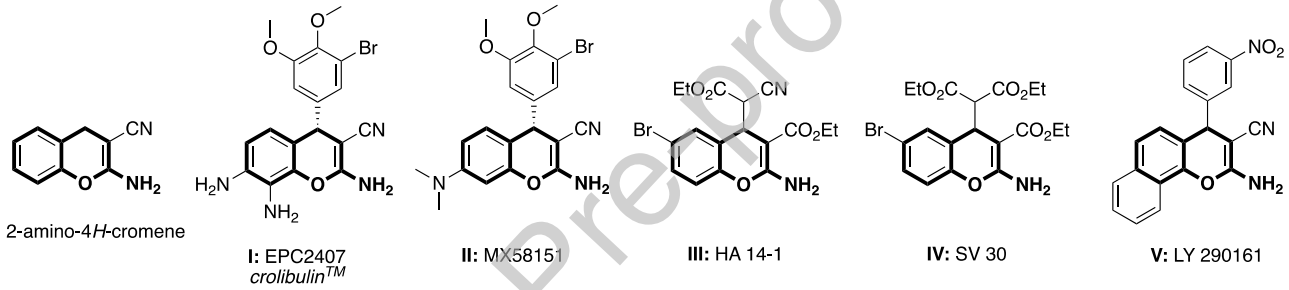

Figure 1: Bioactive 2-amino-4*H*-chromene derivatives reported in literature

Due to the potential pharmacological interest of the 2-amino-4*H*-chromene-3-carbonitrile platform (Figure 1), we were motived 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.

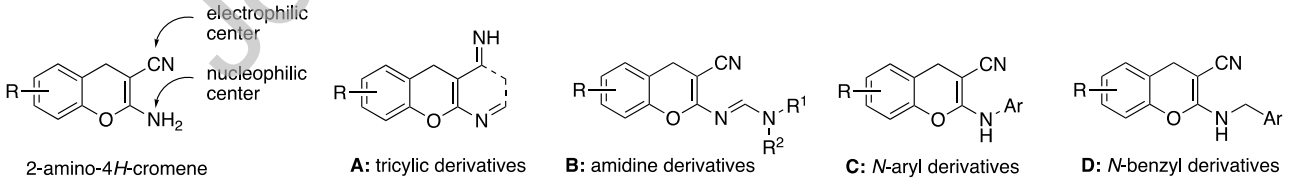

Figure 2: Possibilities of molecular diversity offered by the 2-amino-4*H*-chromene-3-carbonitrile platform

In previous works, we showed that this platform (Figure 2) offers the possibilities to built tricyclic compounds (**A**) [11], amidines (**B**) [12] and *N*-aryl derivatives (**C**) by *trans*-imination of 2-imino-precursors [13]. 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 aera; 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}H NMR spectra were recorded on BRUKER AC 300 Avance III (300 MHz) spectrometer, ^{13}C NMR spectra on BRUKER AC 300 Avance III (75 MHz) spectrometer using respectively $CDCl_3$ or 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: δ 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}H-NMR, ^{13}C-NMR, spectra of compounds **6(a-e)** and **7(a-e)** are provided in Supplementary Material.

*2.1.2 2-Imino-8-methoxy-2H-[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}H NMR (DMSO-d_6) δ : 3.87 (s, 3H, 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-4H-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, ν, cm^{-1}): 1661 (C=C), 2186 (CN), 3332-3456 (NH_2). ^{1}H NMR (DMSO-d_6) δ: 3.43 (s, 2H, 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, NH_2); ^{13}C NMR (DMSO-d_6) δ : 23.8

(CH_2, C-4); 48.8 (CH_3O); 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-4*H*-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^{-2} Torr) at 25°C for 1 h afforded the desired 2-benzylidenamino-8-methoxy-4*H*-chromene-3-carbonitrile **6** as a powder and was analyzed by ^{1}H, ^{13}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^{-1}): 1640 (C=N), 2200 (C≡N). ^{1}H-NMR (DMSO-d_6) δ : 3.83 (s, 2H, CH_2); 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).^{13}C NMR (DMSO-d_6) δ : 26.5 (CH_2, C-4); 56.5 (CH_3O); 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^+, MeOH/CH_2Cl_2 9:1), *m/z*: 313.0950 found (calculated for $C_{18}H_{14}N_2O_2Na$ $[M+Na]^+$ 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^{-1}): 1638 (C=N), 2195 (C≡N). ^{1}H-NMR ($CDCl_3$) δ : 3.11 (s, 6H, 2-CH_3); 3.79 (s, 2H, CH_2); 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* = 8.7Hz, H-1', H-5', Ar); 8.81 (s, 1H, CH=N).^{13}C NMR (100 MHz, $CDCl_3$) δ : 26.9 (CH_2, C-4); 40.1 (2-CH_3); 56.0 (CH_3O); 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^+, MeOH/CH_2Cl_2 9:1), *m/z*: 356.1367 found (calculated for $C_{20}H_{19}N_3O_2Na$ $[M+Na]^+$ 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^{-1}): 1645 (C=N), 2201 (C≡N). ^{1}H-NMR ($CDCl_3$) δ : 3.82 (s, 2H, CH_2); 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).^{13}C NMR ($CDCl_3$) δ : 27.0 (CH_2, C-4); 55.5 (CH_3, C-15'); 56.1 (CH_3, 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^+, MeOH/CH_2Cl_2 9:1) *m/z*: 343.1057 found (calculated for $C_{19}H_{16}N_2O_3Na$ $[M+Na]^+$ 343.10531).

*2.1.4.4 2-[[(E)-(4-Chlorophenyl)methylene]amino]-8-methoxy-4H-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, ν, cm^{-1}): 1615(C=N), 2205 (C≡N). ^{1}H-NMR ($CDCl_3$) δ : 3.83 (s, 2H, 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}C NMR ($CDCl_3$) δ : 27.1 (CH_2, C-4); 56.0 (CH_3O); 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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 347.0556 found $[M+Na]^+$ (calculated for $C_{18}H_{13}N_2O_2{}^{35}ClNa$ $[M+Na]^+$ requires 347.05578).

*2.1.4.5 2-[[(E)-(3-Methoxyphenyl)methylene]amino]-8-methoxy-4H-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, ν, cm^{-1}): 1640(C=N), 2205(C≡N). ^{1}H NMR ($CDCl_3$) δ : 3.84 (s, 2H, 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}C NMR ($CDCl_3$) δ : 27.1 (CH_2, C-4); 55.5 (CH_3, C-14'); 56.1 (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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 343.1056 found (calculated for $C_{19}H_{16}N_2O_3Na$ $[M+Na]^+$ requires 343.10531).

*2.1.5. General procedure for the synthesis of 2-benzylamino-8-methoxy-4H-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}H, ^{13}C NMR, HRMS and centesimal analysis.

*2.1.5.1 2-(Phenylmethyl)amino-8-methoxy-4H-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, ν, cm^{-1}): 2176 (C≡N), 3325 (NH). ^{1}H NMR (DMSO-d_6) δ : 3.42 (s, 2H, CH_2); 3.81 (s, 3H, MeO); 4.39 (d,2H, *J* = 6.4 Hz, CH_2NH); 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}C NMR (DMSO-d_6) δ : 24.6 (CH_2, C-4); 45.0 (CH_2-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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 315.1107 found (calculated for $C_{18}H_{16}N_2O_2Na$ $[M+Na]^+$ requires 315.11095). Anal. Calcd for $C_{18}H_{16}N_2O_2$: 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-4*H*-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, ν, cm^{-1}): 2173 (C≡N), 3337 (NH). ^{1}H NMR ($CDCl_3$) δ : 3.54 (s, 2H, CH_2); 3.89 (s, 3H, MeO); 4.48 (d, 2H, *J* = 5.9 Hz, CH_2NH); 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}C NMR ($CDCl_3$) δ : 24.4 (CH_2, C-4); 40.6 (Me_2N); 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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 358.1526 found (calculated for $C_{20}H_{21}N_3O_2Na$ $[M+Na]^+$ requires 358.1526). Anal. Calcd for $C_{20}H_{21}N_3O_2$: 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-4*H*-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, ν, cm^{-1}): 2172 (C≡N), 3332 (NH). ^{1}H NMR ($CDCl_3$) δ : 3.54 (s, 2H, CH_2); 3.82 (s, 3H, MeO); 3.88 (s, 3H, MeO); 4.51 (d, 2H, *J* = 6.4 Hz, CH_2NH); 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}C NMR ($CDCl_3$) δ : 24.4 (CH_2, C-4); 45.5 (C-11); 51.5 (C-3, C=); 55.3 (CH_3, C-15'); 56.0 (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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 345.1206 found (calculated for $C_{19}H_{18}N_2O_3Na$ $[M+Na]^+$ requires 345.12051. Anal. Calcd for $C_{19}H_{18}N_2O_3$: 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-4*H*-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, ν, cm^{-1}): 12181 (C≡N), 3318(NH). ^{1}H NMR ($CDCl_3$) δ : 3.53 (s, 2H, CH_2); 3.86 (s, 3H, MeO); 4.52 (d, 2H, *J* = 6.3 Hz, CH_2NH); 5.16 (t, 1H, *J* = 5.9Hz, 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}C NMR ($CDCl_3$) δ : 24.3 (CH_2, C-4); 45.2 (C-11); 52.1(C-3, C=); 56.0 (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 (ES^+, MeOH/CH_2Cl_2 9:1) *m/z*: 349.0714 found $[M+Na]^+$ (calculated for $C_{18}H_{15}N_2O_2{}^{35}ClNa$ $[M+Na]^+$ requires 349.07198. Anal. Calcd for $C_{18}H_{15}N_2O_2Cl$: 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-4*H*-chromene-3-carbonitrile **6e** (384 mg, 1.2 mmol) according to the standard procedure which gave **7e** as withe powder, mp = 144-146 °C. IR (KBr, ν, cm^{-1}): 2176 (C≡N), 3362 (NH). ^{1}H NMR ($CDCl_3$) δ : 3.45 (s, 2H, CH_2); 3.72 (s, 3H, MeO); 3.77 (s, 3H, MeO); 4.45 (d, 2H, *J* = 6.2 Hz, CH_2NH); 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}C NMR ($CDCl_3$) δ : 24.3 (CH_2, C-4); 45.9 (C-11); 51.8 (C-3, C=); 55.2 (CH_3, C-14'); 56.0 (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^+, $MeOH/CH_2Cl_2$ 9:1) *m/z*: 345.1214 found (calculated for $C_{19}H_{18}N_2O_3Na$ $[M+Na]^+$ requires 345.12151). Anal. Calcd for $C_{19}H_{18}N_2O_3$: 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 15µM [γ-^{33}P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 µl following the assay described in literature [14]. 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 µg/µl 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 µg/µl of the following peptide: YSPTSPSYSPTSPSYSPTSPSKKKK, as substrate.
- PIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer A with 0.8 µg/µl 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 µg/µl of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate;
- *Ssc*GSK-3α/β (glycogen synthase kinase-3α/β, native, affinity purified from porcine brain extracts, *Sus scrofra domesticus*) was assayed in buffer B (+ 0.15 mg/ml of BSA + 0.23 mg/ml of DTT), with 0.010 µg/µl of GS-1 peptide, a GSK-3-selective substrate (YRRAAVPPSPSLSRHSSPHQSpEDEEE, "Sp" stands for phosphorylated serine).
- *Ssc*CK1δ/ε (porcin casein kinase 1δ/e, native, affinity purified from porcine brain extracts, *Sus scrofra domesticus*) was assayed in buffer A, with 0.022 µg/µl of the following peptide: RRKHAAIGSpAYSITA as CK1-specific substrate.

Buffers:

(A) 60 mM β-glycerophosphate, 30 mM *p*-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM sodium orthovanadate;

(B) 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 µg/ml heparin;

(C) MOPS 25 mM pH 7.5, 10 mM $MgCl_2$.

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.

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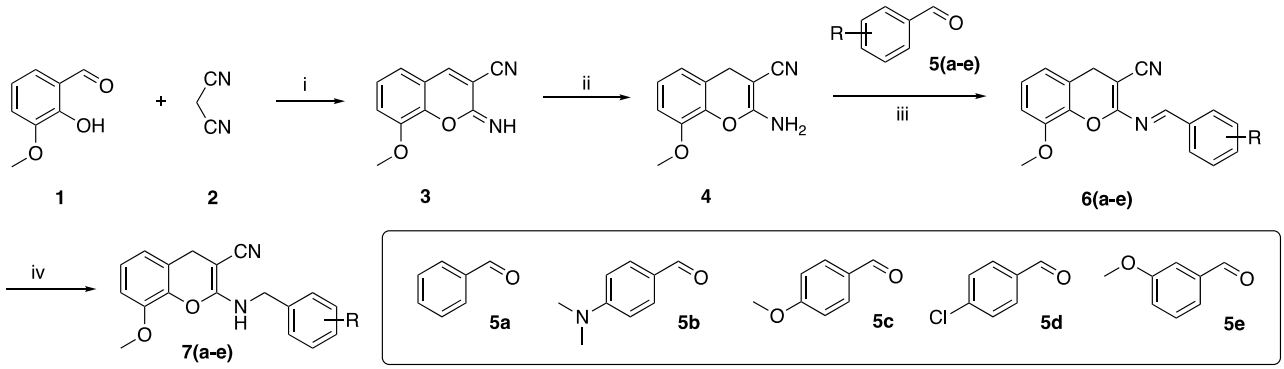
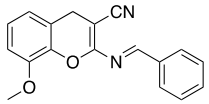


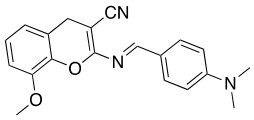
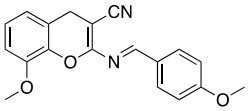
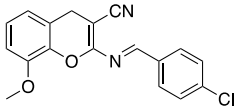
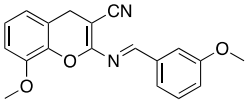
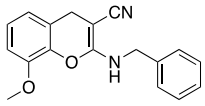
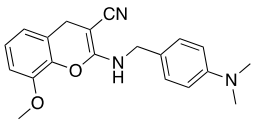
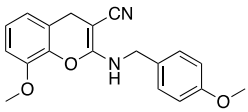
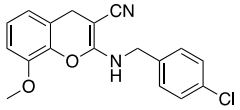
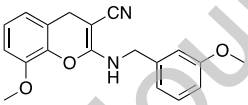
Scheme 1: Synthetic route of the 2-benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile **7(a-e)**. *Reagent and conditions:* i) piperidine 0.5% mol., EtOH, 25°C, 4 h. ii) $NaBH_4$, MeOH, 25°C, 20 min. iii) piperidine, EtOH, 80°C, 5 h. iv) $NaBH_4$, THF, 0°C, 20 min.

The preparation of key intermediate **4** involved a two-step approach developed in our laboratory [11]. 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, $2196 < \nu\ (cm^{-1}) < 2206$), by ^{1}H NMR (N=CH signal, $8.81 < \delta < 8.92$ ppm which is in agreement with a *E*-configuration [15] and ^{13}C NMR (=CH, δ 160.50 ppm). In mass spectrometry analysis (HRMS) the $[M+Na]^+$ 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 **6** and **7**		Yield (%) [a]	Overall yield (%) [b]	$\delta_{CH=}$ of **6** (ppm) [c]		δ_{CH2Ar} of **7** (ppm) [e]	ν_{NH} of **7** (cm^{-1})
6a		70	45	8.88 [d]	162.4 [d]	-	-

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

[a] Yield of isolated product **6** and **7**. [b] Overall yield of **6** and **7** calculated from compound **3**. [c] ^{1}H NMR and ^{13}C NMR in $CDCl_3$ solution. [d] ^{1}H NMR and ^{13}C NMR in DMSO-d_6 solution with TMS as internal reference. [e] ^{1}H NMR in $CDCl_3$ 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 ^{1}H, ^{13}C NMR, FTIR, HRMS and centesimal analysis. Examination of their ^{1}H NMR spectra in DMSO-d_6 solution showed that the CH_2Ar signal is located in the range $4.38 < \delta < 4.51$ ppm and in the IR spectrum, important bands of absorption were observed between 3318 and 3361 cm^{-1} which can be attributed to the N-H stretching frequencies (Table 1).

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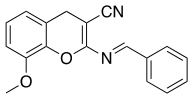
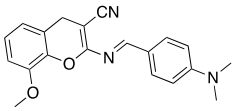
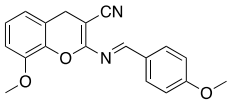
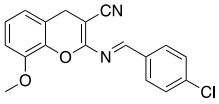
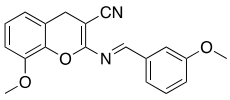
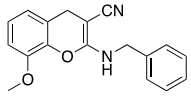
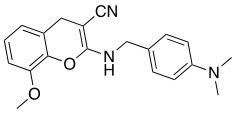
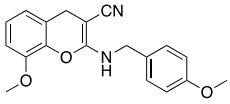
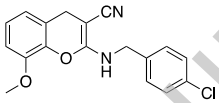
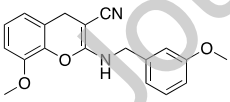
3.2 Physicochemical properties and *in silico* ADME studies

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^2)	HBA	HBD	RB	Fract. Csp^3	LV
6a		290.32	3.26	-3.89	54.61	4	0	3	0.11	0
6b		361.44	3.95	-4.60	57.85	4	0	6	0.27	0
6c		320.34	3.27	-3.95	63.84	5	0	4	0.16	0
6d		324.76	3.80	-4.48	54.61	4	0	3	0.11	0
6e		320.34	3.27	-3.95	63.84	5	0	4	0.16	0
7a		293.33	3.13	-4.29	54.28	3	1	4	0.17	0
7b		363.45	3.75	-5.00	57.62	3	1	7	0.32	0
7c		322.36	3.10	-4.36	63.51	4	1	5	0.21	0
7d		326.78	3.67	-4.89	54.28	3	1	4	0.17	0
7e		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 aera; 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 [16] server platform [17]. These physicochemical properties were evaluated based on Lipinski's rules [18], 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 aera (tPSA), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) [19], number of rotatable bonds (RB), the fraction of Csp^3 and drug-likeliness (violation of the 5 Lipinski rules) completed this *in silico* analysis (Table 2).

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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*-NEt_2) and to **6d** (R = *p*-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 aera (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 $Å^2$ 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) [20] for the following requirements: (a) a molecular weight < 500 g/mole; (b) hydrogen bond donors HBD < 5; (c) hydrogen bond acceptors HBA < 10 [21]; (d) Log P < 4.15 and (e) tPSA ≤ 140 $Å^2$. This means that these compounds should have efficient absorption and good oral potential bioavailability [22].

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 (Lipophility): -0.7 < Log P < +5.0; SIZE: 150 g/mol < MW < 500 g/mol; POLAR (Polarity): 20 $Å^2$ < tPSA < 130 $Å^2$; INSOLU (Insolubility): -6 < Log S < 0; INSATU (Insaturation): 0.25 < Fraction Csp^3 < 1; FLEX (Flexibility): 0 < Number rotatable bonds < 9.

6a	**6b**	**6c**	**6d**	**6e**
%Abs: 55	%Abs: 55	%Abs: 55	%Abs: 55	%Abs: 55
7a	**7b**	**7c**	**7d**	**7e**
%Abs: 55	%Abs: 55	%Abs: 55	%Abs: 55	%Abs: 55

Using the SwissADME server [17], 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 2nd 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: *Hs*CDK5-p25 (*Homo sapiens* cyclin-dependant kinase 5-p25) [23]; *Hs*CDK9/cyclin T (*Homo sapiens* cyclin-dependant kinase 9) [24]; *Hs*Pim1 (*Homo sapiens* Pim1, proto-oncogene, serine/threonine kinase) [25]; *Hs*Haspin (*Homo sapiens* Haspin, a mitotic serine/threonine protein kinase); *Ssc*GSK-3α/β (*Sus scofra domesticus* glycogene synthase kinase-3α/β) [26] and *Ssc*CK1δ/ε (*Sus scofra domesticus* casein kinase1δ/ε) [27]. All the selected enzymes presented interest for protein phosphorylation of serine, threonine and tyrosine residues in many regulatory mechanisms of cells. Deregulation of these protein kinases is mainly responsible of human diseases [28].

In Table 4 are reported the results of *in vitro* kinase assays with the rate (%) of residual activities at 10 μM and 1 μM. 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 μM and 1 μM). 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 μM and at 1 μM, so the tested compounds have a marked effect on the catalytic activity.

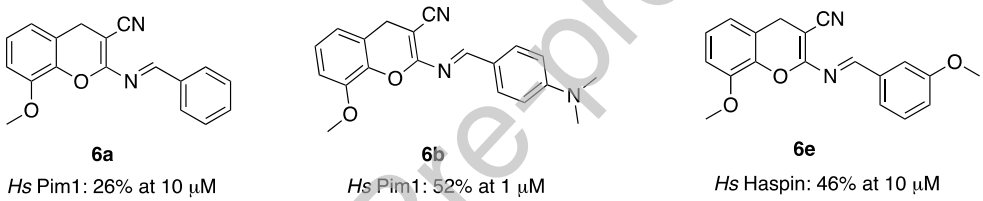

Figure 3: 2-Benzylidenamino-8-methoxy-4*H*-chromene-3-carbonitrile **6(a,b)** and **6e** which are bioactive against protein kinases Pim1 and Haspin. *Hs*, *Homo sapiens*.

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 μM) and **6b** (55% rate at 1 mM); for Haspin, only the compound **6e** is active (46% rate at 10 μM).

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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 µmol/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 domesticus*.

Compound	Concentration (µM)	CDK5-p25	CDK9/ cyclin T	Pim1	Haspin *	*Ssc*GSK 3α/β	*Ssc*CK1δ/ε
6a	10	≥100	77	26	79	83	63
	1	≥100	104	99	98	77	≥100
6b	10	≥100	88	55	71	92	49
	1	99	93	52	80	89	75
6c	10	94	≥100	≥100	86	88	≥100
	1	100	≥100	≥100	96	83	69
6d	10	97	79	60	68	65	72
	1	95	72	≥100	≥100	80	59
6e	10	≥100	88	73	46	71	≥100
	1	≥100	83	88	69	73	≥100
7a	10	84	≥100	81	≥100	≥100	97
	1	≥100	96	86	≥100	100	97
7b	10	≥100	85	100	≥100	93	≥100
	1	≥100	≥100	87	97	99	≥100
7c	10	≥100	88	73	99	84	≥100
	1	≥100	84	74	66	97	92
7d	10	97	89	68	71	91	86
	1	≥100	88	≥100	≥100	≥100	74
7e	10	≥100	79	87	≥100	75	59
	1	≥100	84	76	59	75	83

NB: ≥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,

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log S, tPSA, HBA, HBD, fraction Csp^3, 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.

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Supplementary material

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

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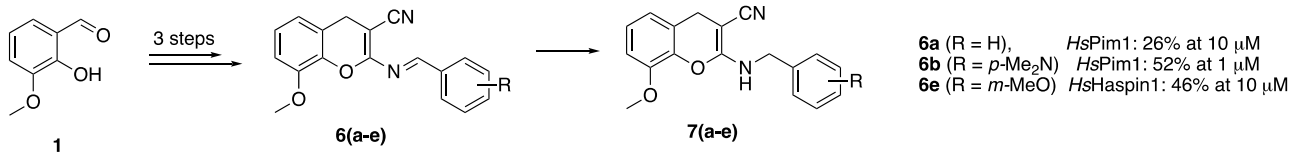
Graphical abstract



1 **6(a-e)** **7(a-e)**

6a (R = H), *Hs*Pim1: 26% at 10 μM
6b (R = *p*-Me_2N) *Hs*Pim1: 52% at 1 μM
6e (R = *m*-MeO) *Hs*Haspin1: 46% at 10 μM

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Highlights

- 2-Benzylamino-8-methoxy-4*H*-chromene-3-carbonitrile
- Reductive amination
- Lipinski's descriptors
- Protein kinase
- *Hs*Pim1 inhibitor

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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

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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.

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