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Ali Bouattour, Mehdi Fakhfakh, Souhir Abid, Ludovic Paquin, Rémy Le Guével, et al.. Synthesis of New 2-Phenylamino-4H-chromene-3-carbonitrile Derivatives and Their Effects on Tumor Cell Lines and against Protein Kinases. *International Journal of Organic Chemistry*, 2020, 10 (02), pp.88-103. 10.4236/ijoc.2020.102006 . hal-03869130v1

HAL Id: hal-03869130

<https://hal.science/hal-03869130v1>

Submitted on 10 Feb 2021 (v1), last revised 24 Nov 2022 (v2)

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Synthesis of new 2-phenylamino-4*H*-chromene-3-carbonitrile derivatives and their effects on tumor cell lines and against protein kinases

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Abstract

The synthesis of 2-phenylimino-4*H*-chromene-3-carbonitriles **6(a-d)** in good overall yields using an efficient and practical methodology in 3 steps has been implemented in this present work. The first step was an heterocyclization between 2-hydroxybenzaldehyde **1** and propanedinitrile **2** which produced 2-iminocoumarin **3** which was submitted to nitrogen/nitrogen displacement in the presence of aromatic primary amine **4**. In the third step, reduction of **5** led to the desired 2-phenylimino-4*H*-chromene-3-carbonitriles **6**. Compounds **5(a-d)** and **6(a-d)** were evaluated for their potential *in vitro* cytotoxicity against six selected tumor cell lines (Huh7-D12, Caco2, MDA-MB231, HCT 116, PC3 and NCI-H727) and tested for their protein kinase inhibition on eight selected protein kinases. Among them, compounds **5c** and **6b** exhibited inhibition on *HsCK1ε* (**5c**: 44% and **6b**: 42% at 1 μM) and **5c** for cytotoxicity on PC3 cell lines (63% at 25 μM).

Key words:

iminocoumarin, 2*H*-[1]benzopyran, 2-imino-2*H*-[1]benzopyran, 4*H*-chromene, 2-amino-4*H*-chromene, nitrogen/nitrogen displacement, protein kinase inhibition, cytotoxicity.

1. Introduction

During the two last decades, the 2-amino-4*H*-chromene moieties (Figure 1) emerged to be a promising platform in new chromene and benzo chromene derivatives, which exhibit a wide range of biological and pharmacological activities. For examples, Crolibulin™ (EPC 2407) (**I**) was currently in phase I/II of clinical trials in 2016 for the treatment of aggressive and advanced solid tumors [1-4]. During the randomized phase II trial (NTC01240590) [5], the authors compared the activity of the combination of Crolibulin™ plus cisplatin with cisplatin alone on a maximum of 40 enrolled patients with a focus on anaplastic thyroid cancer (ATC).

MX 58151 (**II**) or 2-amino-4-(3-bromo-4,5-dimethoxyphenyl)-4*H*-chromene-3-carbonitrile was identified as a tubulin inhibitor [6]. It induced apoptosis with an EC₅₀ of 50 nM and inhibited cell growth with a GI₅₀ of 37 nM in T47D breast cancer cells [7]. HA 14-1 (**III**) is another small molecule and nonpeptidic ligand of Bcl-2 surface pocket discovered by using the *de novo* computer-aided design strategy based on the predicted structure of Bcl-2 protein [8]. *In vitro* binding studies of HA 14-1 (**III**) against a set of Jurkat cells confirmed inhibition of Bcl-2 protein [9, 10] and the authors of this study demonstrated that the 6-bromo on (**III**) is not essential for its bioactivity and the 6-position can accommodate a variety of alkyl and aryl functional groups [11]. SV30 or ethyl [2-amino-6-bromo-4-diethylmalonate]-4*H*-chromene-3-carboxylate (**IV**), an analogue of pro-apoptotic molecule HA 14-1 (**III**), was explored in combination with lipid nanocapsules (LNCs) on F98 cells to improve its biological activities [12]. The authors demonstrated that SV30-LNCs were able to trigger cell death together with a potentiation of the mitochondrial membrane potential decrease. The naphthopyran LY 290181 (**V**) [2-amino-4-(pyridyl)-4*H*-naphtho[1,2-*b*]pyran-3-carbonitrile] was identified in 1997 as a potent antiproliferative compound blocking cells in the G₂/M phase of the cell cycle associated to action on microtubules [13, 14]. Finally, it's interesting to note that a QSAR (Qualitative Structure Activity Relationship) analysis was developed with novel 3D-descriptors using Triplets Of Pharmacophoric Points (TOPP) on 80 virtual apoptosis inducing 3-amino-4-aryl-4*H*-chromene-3-carbonitriles [15], the underlying idea of this work was to optimize the pharmacological and pharmacokinetic properties *via* QSAR models prior to synthesis.

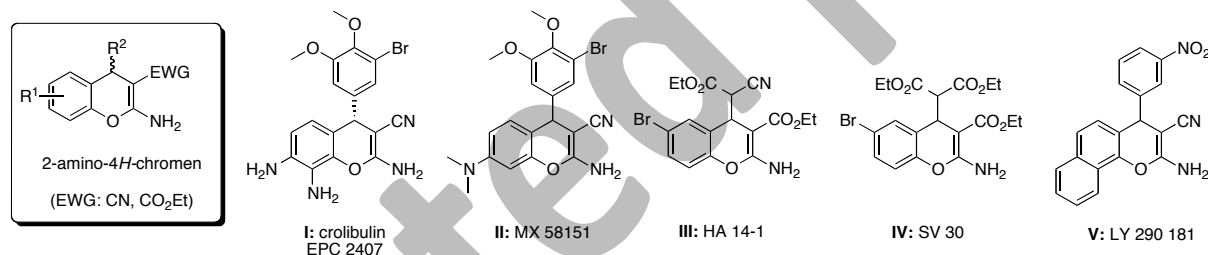


Figure 1: Structure of bioactive 2-amino-4*H*-chromene derivatives.

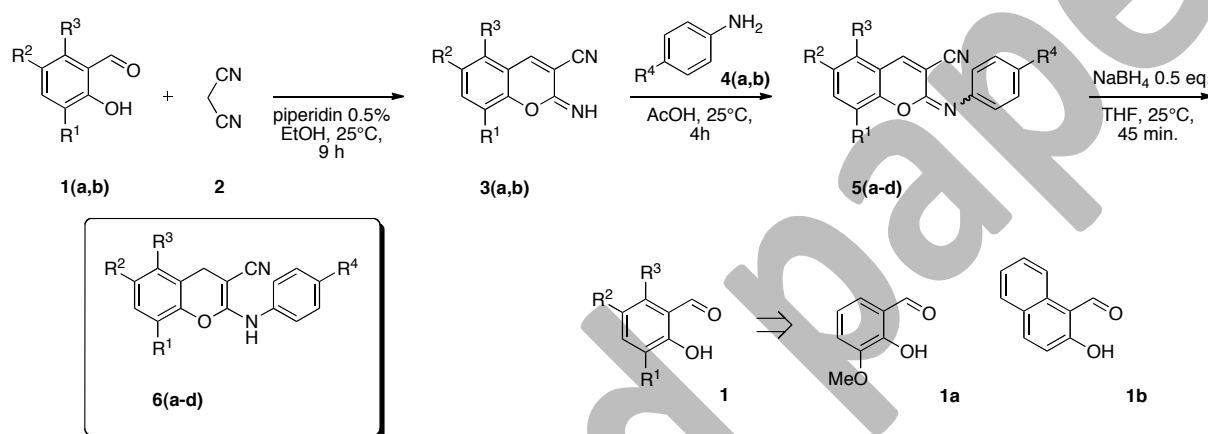
As part of our program aimed at developing new methods, new building-blocks and platforms for the preparation of heterocyclic compounds showing potential biological properties for central nervous system (CNS) (Alzheimer's disease and Down syndrome) [16, 17], or for cancer (calcium ion channel inhibitors in cancer) [18], we were motivated in this work by the synthetic development of some new 2-amino-4*H*-[1]-chromene-3-carbonitriles without aryl substituent in C-4 position but with a phenyl fragment on the 2-amino function, to evaluate their biological activities on protein kinases (PKs) and also their anti-proliferative activities on tumor cell lines.

2. Results and discussion

2.1. Chemistry

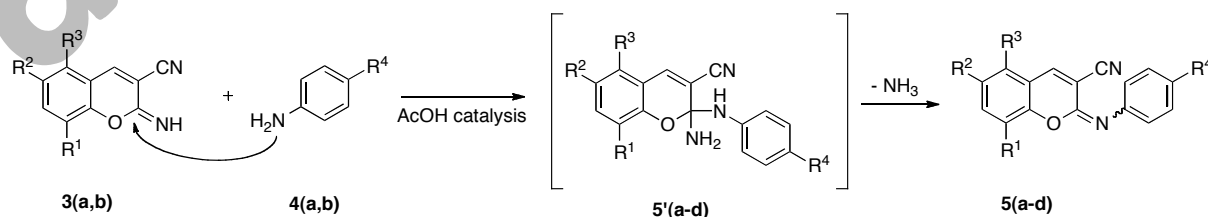
For this project, the desired 2-*N*-phenylamino-4*H*-chromene-3-carbonitriles **6(a-d)** were prepared only in three steps (Scheme 1). The first step involved synthesis of iminocoumarins **3(a,b)** or 2-imino-2*H*-[1]-benzopyran-3-carbonitriles and protocol of this classical heterocyclization was developed in our laboratory [19-21]. The reaction was realized from an equimolecular mixture of 2-hydroxybenzaldehyde **1** (**1a**: 2-hydroxy-3-methoxybenzaldehyde or *o*-vanillin, **1b**: 2-hydroxynaphthaldehyde) and propanedinitrile **2** using 0.5% of piperidine in ethanol at room temperature. After a reaction time of 9 hours, the volatile compounds were eliminated *in vacuo* and we obtained these starting iminocoumarins in good yields (**3a**: 85%

[19], **3b**: 90% [22]). For the second step, the transformation of the 2-imino-2*H*-[1]-benzopyran-3-carbonitriles **3(a,b)** into 2-*N*-phenylimino-2*H*-[1]-benzopyran-3-carbonitriles **5(a-d)** is the key step in our approach for introduction of molecular diversity in this C-2 position. In a preliminary approach, we studied this nitrogen/nitrogen displacement with volatile primary aliphatic amine **4** (from 1 to 2 equivalents) using solvent-less reaction conditions [23] in a mono-mode microwave cavity (Monowave® 300 Anton-Paar apparatus operating at a frequency of 2.45 GHz from 0 to 800 Watt). Initial attempts to obtain a good reproducibility for this nitrogen/nitrogen displacement with primary aliphatic amine **4** involving modification of the reaction conditions (reaction time range: 15-60 min., ratio of reagents **3** / **4**: from 1 to 2, reaction temperature, use of non-polar or polar solvents) were unsuccessful. Therefore, we decided to abandon this solvent-less microwave approach.



Scheme 1: Route used for the synthesis of 2-*N*-phenylamino-4*H*-chromene-3-carbonitrile derivatives **6(a-d)**.

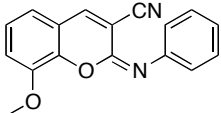
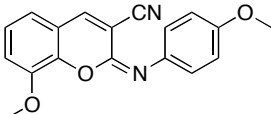
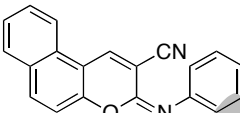
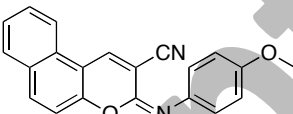
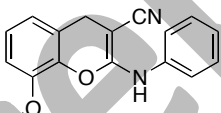
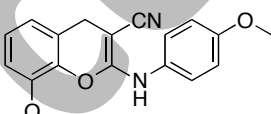
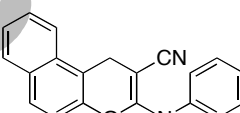
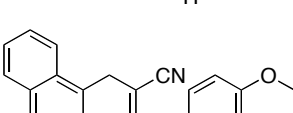
On the other hand, the use of an aromatic primary amine **4(a,b)** associated with an acidic catalysis was much fruitful. Indeed, when this reaction was carried out in glacial acetic acid with an equimolecular mixture of **3** and **4** at room temperature, we observed that after 4 hours, the desired compound **5** obtained by nitrogen/nitrogen displacement (Scheme 2) becomes insoluble in the reaction medium. Thus recovery of this insoluble material by filtration on a Buchner funnel followed by washing with deionized water allows us to get very easily the four desired 2-*N*-phenylimino-2*H*-[1]-benzopyran-3-carbonitriles **5(a-d)** in yields ranging from 55 to 80% (Table 1). ¹H NMR control in DMSO-*d*₆ of **5(a-d)** after filtration showed that no need of supplementary purification and it can be used as it in the next step. Finally for the third step involving transformation of 2-*N*-phenylimino-2*H*-[1]-benzopyran-3-carbonitriles **5(a-d)** into 2-*N*-phenylamino-4*H*-chromene-3-carbonitriles **6(a-d)**, we used an experimental protocol developed in our laboratory [19] but only changing the nature of the solvent reaction (THF vs MeOH) and the reaction temperature. From 0.5 equivalent of sodium borohydride in THF at 25°C during 45 min., the desired compounds **6(a-d)** were obtained in good yields (Table 1) after purification (81-89%).



Scheme 2: A plausible suggested mechanism for nitrogen/nitrogen displacement *via* a nucleophilic attack of amino group of **4** to C-2 imino function of **3**.

Before exploring their potential biological activities, the four products **5(a-d)** and their four reduced derivatives **6(a-d)** were characterized by ^1H , ^{13}C NMR, HRMS and FTIR. In the IR spectrum, the presence of carbonitrile function in C-3 position was detected at 2227 - 2229 cm^{-1} for **5(a-d)** and at 2189 - 2195 cm^{-1} for **6(a-d)** (Table 1). The C-4 imino functions of **5(a-d)** were analyzed at 1650 - 1662 cm^{-1} . Important absorption bands were observed at 3278 - 3320 cm^{-1} for the NH stretching frequencies of **6(a-d)**. In ^1H NMR spectrum of compounds **6(a-d)**, a signal located at $3.54 < \delta < 3.98$ ppm is in agreement with the H-4 methylene fragment. In ^{13}C NMR spectrum, the signal for the CH_2 fragment in position C-4 of compounds **6(a-d)** is located at $22.4 < \delta < 25.3$ ppm. For HRMS analysis, the $[\text{M}+\text{Na}]^+$ molecular ion signal for all products **5** and **6** were obtained as base signal.

Table 1: Results obtained for the preparation of 2-*N*-phenylimino-2*H*-[1]-benzopyran-3-carbonitrile **5(a-d)** and 2-*N*-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)**.

Compound	Yield (%) ^a	mp (°C)	IR (en cm^{-1})		
			$\nu(\text{C}=\text{N})$	$\nu(\text{nitrile})$	$\nu(\text{NH})$
5a 	60	174-176	1650	2227	-
5b 	70	180-182	1650	2227	-
5c 	55	196-198	1662	2229	-
5d 	80	232-234	1650	2229	-
6a 	81	122-124	-	2195	3320
6b 	89	148-150	-	2191	3232
6c 	84	188-190	-	2193	3278
6d 	85	200-202	-	2189	3235

^a Yield of isolated product.

2.2. Biology

For the second part of this project related to 2-*N*-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)** and their precursors **5(a-d)**, we have been interested to exploring their potential biological properties, particularly their impact on protein kinase inhibition activity and their cytotoxic character against various tumor cell lines.

Table 2: Effects of 2-phenylimino-2*H*-benzopyran-3-carbonitrile **5(a-d)** and 2-*N*-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)** on the catalytic activity of eight protein kinases.

Primary screening (% of residual activity at 10 μM and 1 μM)									
Compound	Conc. (μM)	CDK5/p25	CDK9/cyclin T	CK1ε	GSK3β	Haspin	PIM1	CLK1	DYRK1A
5a	10	≥ 100	96	60	79	55	96	80	71
	1	98	76	65	82	91	84	88	99
5b	10	98	84	78	85	68	97	78	≥ 100
	1	95	61	52	90	≥ 100	99	87	85
5c	10	98	72	63	78	60	72	90	90
	1	94	57	44	72	45	81	80	58
5d	10	91	51	38	65	34	79	81	82
	1	94	54	57	82	51	78	75	57
6a	10	86	62	58	66	46	52	51	34
	1	≥ 100	60	63	74	67	84	83	71
6b	10	76	57	47	71	53	68	74	65
	1	86	50	42	65	90	92	75	67
6c	10	80	43	46	55	38	72	81	74
	1	89	54	59	64	71	80	71	78
6d	10	87	38	39	31	31	76	61	≥ 100
	1	89	63	67	78	66	77	71	88

NB: ≥ 100, indicates that the compound cannot inhibit the enzymatic activity at the tested concentration

	The compound has an interest on the catalytic activity because the rate is less than or close to 50%
	The compound has a marked effect on the catalytic activity because the rate is less than 50%

The new compounds **5** and **6** were evaluated on eight different *in vitro* kinase assays. The selected enzymes are very important in protein phosphorylation of serine, threonine and tyrosine residues, which are connected, in many cellular regulatory mechanisms; dysfunction of protein kinase activities is in many cases responsible of human diseases. The protein kinases used for these *in vitro* assays are respectively *HsCDK5-p25* (*Homo sapiens* cyclin-dependent kinase 5p-25) [24], *HsCDK9/cyclin T* (*Homo sapiens* cyclin T dependent kinase 9 protein) [25], *SscCK1ε* (casein kinase 1ε, from porcine brain) [26], *SscGSK3β* (glycogen synthase kinase-3β, from porcine brain) [27], *HsPim1* (*Homo sapiens* Pim1 proto-oncogene,

serine/threonine kinase) [28], *Mm*CLK1 (from *Mus musculus*, Dual specificity protein kinase) [29] and *Rn*DYRK1A-kd (*Rattus norvegicus*, Dual specificity tyrosine phosphorylation regulated kinase 1A) [30].

Results for these *in vivo* kinase assays are reported in Table 2 with the rate (%) of residual activities at 10 μ M and 1 μ M. These results showed three categories of compounds. The first concerns compounds with no significant protein kinase activity (rate > 60-70% at 10 μ M and 1 μ M). The second category integrated compounds with moderate interest because the rate is less than or close to 50% and for the third category; the rate is less than 50% at 10 μ M and 1 μ M. In this last one, the tested compounds **5** or **6** have a marked effect on the catalytic activity. Examination of results given in table 2 showed that many compounds (Figure 2) exhibited inhibitory activity on *Hs*CK1 ϵ , *Hs*Haspin, *Hs*CDK9/cyclin T. This concerned respectively and particularly *Hs*CK1 ϵ with **5c** (41% at 1 μ M), **5d** (57% at 1 μ M) and **6c** (46% at 10 μ M and 59% at 1 μ M). For *Hs*Haspin, the better result was obtained with **5c**: 45% at 1 μ M. For *Rn*DYRK1A and *Hs*GSK3 β , good residual activities were observed only at 10 μ M for **6a** (*Rn*DYK1A: 34%) and **6d** (*Hs*GSK3 β : 31%).

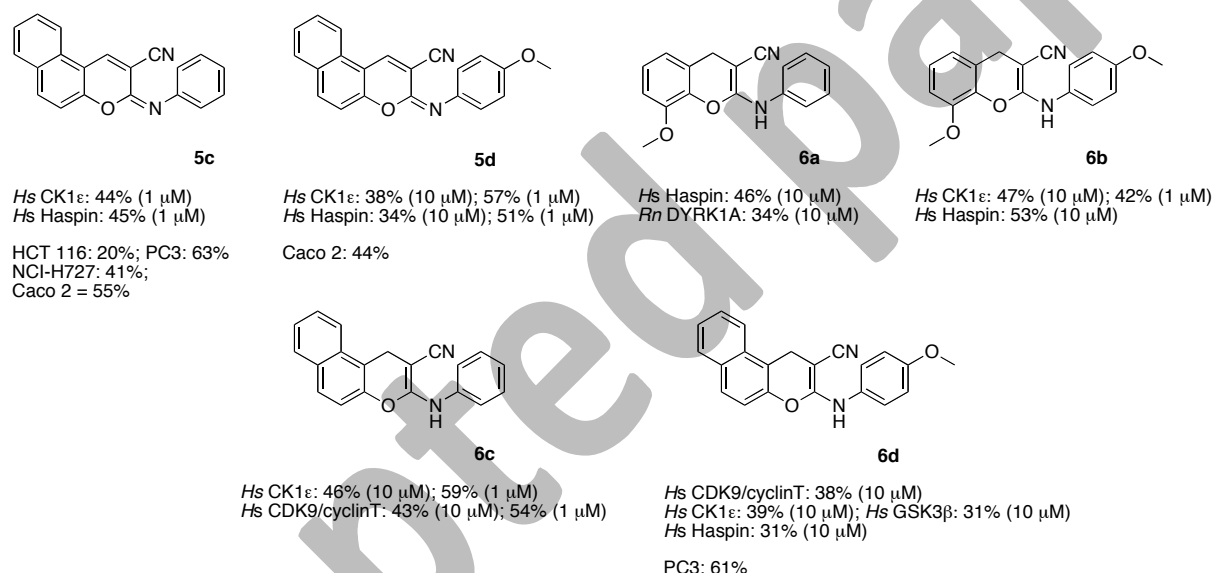


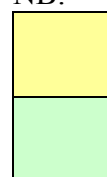
Figure 2: 2-Phenylimino-2*H*-[1]-benzopyran-3-carbonitrile **5(c, d)** and 2-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)** which are bioactive on tumor cell lines or against protein kinases

Pursuing this biological exploration, the 2-phenylimino-2*H*-[1]-benzopyran-3-carbonitrile **5(a-d)** and 2-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)** were also subjected for their *in vitro* cytotoxic potential on six selected human cancer cells which are respectively: Huh7 for hepatocellular carcinoma, Caco2 for colorectal adenocarcinoma, MDA-MB231 for human breast adenocarcinoma, HCT 116 for colon carcinoma, PC3 for human Caucasian prostate adenocarcinoma and NCI-H727 for human lung non-small cell carcinoma. For each tumoral cell line, the % of cell survival was measured at a single dose of 25 μ M (after 48 h) in triplicate. Roscovitine, doxorubicin and taxol were used as references for positive control. It can be observed from these primary screening results reported in Table 3 that only two compounds exhibited antiproliferative activities in tumoral cell lines. This concerns **5c**, which exhibited cytotoxic effect on HCT 116 (20% of survival), PC3 (63%) and NCI-H727 (41%). The presence of a methoxy group on the 2-phenylimino moiety in **5d** increase the percentage of survival, excepted on Caco2 (44%). For compound **6d**, which is the reduced derivative of **5d**, it showed cytotoxicity against PC3 (61%).

Table 3: Antiproliferative activity of 2-phenylimino-2*H*-benzopyran-3-carbonitrile **5(a-d)** and 2-phenylamino-4*H*-chromene-3-carbonitrile **6(a-d)** on six representative tumor cell lines.

Compound	% of survival measured at 25 μ M (after 48 h using a single dose, triplicate)					
	Huh7 D12	Caco 2	MDA-MB231	HCT 116	PC3	NCI-H727
5a	98	100	111	115	88	109
5b	95	89	110	64	83	79
5c	71	55	78	20	63	41
5d	61	44	65	54	60	54
6a	103	92	103	97	95	91
6b	83	98	118	119	87	101
6c	79	87	90	99	85	98
6d	86	90	82	74	61	91
DMSO	100	100	100	100	100	100
Roscovitine	45	21	38	7	19	9
Doxorubicin	64	79	46	23	37	31
Taxol	50	78	50	9	26	26

NB:



The compound has antiproliferative activity on tumor cell but the survival rate is less than or close to 50%

The compound has a marked antiproliferative activity on tumor cell and the survival rate is less than 50%

3. Conclusion

This preliminary study described a practical approach to 2-*N*-phenylamino-4*H*-chromene-3-carbonitriles **6(a-d)** in three steps. The key step in this methodology is the nitrogen/nitrogen displacement between 2-imino-2*H*-[1]-benzopyran-3-carbonitriles **5(a-d)** and aromatic primary amines **4(a,b)** in acetic medium on the 2-imino function. Insolubility of compounds **5(a-d)** in the acetic acid mixture facilitated its separation by simple filtration, which offers a practical, attractive protocol and extension to a wide variety of aromatic primary amine **4** is possible. The explorations of biological activities were successively performed on a panel of six tumoral cell lines and against eight protein kinases. Among the bioactive compounds, **5c** and **6b** turned out to be interesting because they present a good percentage of residual activities at 1 μ M for HsCK1 ϵ . **5c** showed also a marked antiproliferative activity on HCT 116 (20% of survival). The present work is the starting point of a new longer program through a next structure activity relationship (RSA) study for a complete identification of a better promising anticancer agent bearing a 2-amino-4*H*-chromene platform.

4. Experimental section

4.1. Chemistry

General information: Solvents were evaporated with a BUCHI rotary evaporator. All reagents and solvents were purchased from Acros Fisher, Sigma-Aldrich Chimie, and Fluka France and were used without further purification. ^1H NMR spectra were recorded on

BRUKER Avance 300 (300 MHz) spectrometer and ^{13}C NMR spectra on BRUKER Avance 300 (75 MHz) spectrometer. The high resolution mass spectra (HRMS) were recorded in positive mode using direct Electrospray infusion, respectively on a Waters Q-ToF 2 or on a Thermo Fisher Scientific Q-Exactive spectrometers at the "Centre Régional de Mesures Physiques de l'Ouest" platform and centesimal analysis of the final compounds was performed on a microanalyzer Thermo Scientific Flash EA1112 CHNS/O at the CRMPO platform, ScanMAT UMS CNRS 2001, Rennes, France). IR spectra were registered on a Jasco FT-IR 420 spectrophotometer using KBr pellets. Melting points were determined on a Kofler melting point apparatus and were uncorrected. 2-Imino-2*H*-[1]-benzopyran-3-carbonitrile **3(a,b)** were synthesized according to procedure described in literature [20, 22].

Standard procedure for the synthesis of 2-N-phenylimino-2H-[1]-benzopyran-3-carbonitrile 5(a-d).

To a stirred solution of 2-imino-2*H*-1-benzopyrane-3-carbonitriles **3** (1 mmol) in glacial acetic acid (2 ml) was added the primary amine **4** (1 mmol). The resulting mixture was vigorously stirred (500 rpm) at room temperature during 4 hours. The desired product **5** was collected by filtration on a Buchner funnel (porosity No. 4) and washed with deionized water (3 x 10 ml). The desired 2-phenylimino-2*H*-1-benzopyrane-3-carbonitriles **5** was dried under high vacuum (10^{-2} torr) at 25 °C for 1 h to give a powder and then used without purification.

8-Methoxy-2-N-phenylimino-2H-[1]-benzopyran-3-carbonitrile (5a)

Yield = 60%. Yellowish powder, mp = 174-176°C. IR (cm^{-1}): $\nu = 1650$ (C=N), 2227 (C≡N); ^1H NMR (300 MHz, DMSO- d_6) δ : 3.81 (s, 3H, OCH₃), 7.22 (m, 4H, H₅, H₆, H₇, H₄), 7.38 (d, $^3J = 3$ Hz, 4H, H₂, H₃), 8.40 (s, 1H, H₄); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 56.5 (OCH₃), 105.7 (C₃), 115.0 (C≡N), 117.2 (C₇), 118.2 (C₁₀), 120.5 (C₅), 123.7 (C₂), 124.7 (C₄), 124.9 (C₆), 128.6 (C₃), 142.3 (C₉), 144.1 (C₁), 144.7 (C₈), 146.4 (C₂), 146.5 (C₄). HRMS, m/z : 299.0792 found (calculated for C₁₇H₁₂N₂O₂Na [M+Na]⁺ requires 299.0792). Anal. Calcd for C₁₇H₁₂N₂O₂: C, 73.90; H, 4.38; N, 10.14. Found C, 73.92; H, 4.40; N, 10.15.

8-Methoxy-2-(4-methoxy-phenylimino)-2H-[1]-benzopyran-3-carbonitrile (5b)

Yield = 70%. Reddish powder, mp = 180-182°C. IR (cm^{-1}): $\nu = 1650$ (C=N), 2227 (C≡N); ^1H NMR (300MHz, DMSO- d_6) δ : 3.79 (s, 3H, H₄), 3.89 (s, 3H, OCH₃), 6.95 (d, $^3J = 9$ Hz, 2H, H₃), 7.15 (d, $^3J = 9$ Hz, 1H, H₇), 7.23 (t, $^3J = 9$ Hz, 1H, H₆), 7.31 (d, $^3J = 9$ Hz, 1H, H₅), 7.51 (d, $^3J = 9$ Hz, 2H, H₂), 8.32 (s, 1H, H₄); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 55.2 (C₄), 56.5 (OCH₃), 106.2 (C₃), 113.8 (C₃), 115.1 (C≡N), 116.8 (C₇), 118.2 (C₁₀), 120.3 (C₅), 124.9 (C₆), 126.1 (C₂), 136.7 (C₁), 142.3 (C₉), 143.4 (C₈), 145.4 (C₂), 146.4 (C₄), 156.9 (C₄). HRMS, m/z : 329.0899 found (calculated for C₁₈H₁₄N₂O₃Na [M+Na]⁺ requires 329.0897). Anal. Calcd for C₁₈H₁₄N₂O₃: C, 70.58; H, 4.61; N, 9.15. Found C, 70.60; H, 4.62; N, 9.15.

2-N-Phenylimino-3H-naphtho[2,1-b]pyran-2-carbonitrile (5c)

Yield = 55%. Yellowish powder, mp = 196-198°C. IR (cm^{-1}): $\nu = 1662$ (C=N), 2229 (C≡N); ^1H NMR (300 MHz, CDCl₃) δ : 7.20 (t, $^3J = 6$ Hz, 1H, H₄), 7.23 (d, $^3J = 6$ Hz, 1H, H₈), 7.34 (d, $^3J = 6$ Hz, 2H, H₂), 7.41 (t, $^3J = 6$ Hz, 2H, H₃), 7.59 (t, $^3J = 6$ Hz, 1H, H₅), 7.74 (t, $^3J = 6$ Hz, 1H, H₅), 7.90 (d, $^3J = 6$ Hz, 1H, H₆), 8.00 (d, $^3J = 6$ Hz, 1H, H₇), 8.14 (d, $^3J = 6$ Hz, 1H, H₅), 8.55 (s, 1H, H₄); ^{13}C NMR (75 MHz, CDCl₃) δ : 106.2 (C₃), 111.7 (C≡N), 115.1 (C₈), 116.3 (C₁₀), 121.0 (C₅), 123.1 (C₂), 124.9 (C₆), 126.4 (C₅), 128.8 (C₄), 128.8 (C₃), 129.2 (C₄), 129.3 (C₇), 130.3 (C₆), 135.3 (C₆), 140.4 (C₅), 144.6 (C₁), 144.8 (C₂), 153.8 (C₉). HRMS, m/z : 319.0840 found (calculated for C₂₆H₁₂N₂O₂Na [M+Na]⁺ requires 319.0842). Anal. Calcd for C₂₆H₁₂N₂O: C, 81.07; H, 4.08; N, 9.45. Found C, 81.08; H, 4.08; N, 9.44.

2-(4-Methoxyphenylimino)-3H-naphtho[2,1-b]pyran-2-carbonitrile (5d)

Yield = 80%. Reddish powder, mp = 232-234°C. IR (cm⁻¹): ν = 1650 (C=N), 2229 (C≡N); ¹H NMR (300 MHz, CDCl₃) δ : 3.87 (s, 3H, H_{4''}), 6.95 (d, ³J = 9 Hz, 2H, H_{3'}), 7.31 (d, ³J = 6 Hz, 1H, H₈), 7.44 (d, ³J = 9 Hz, 2H, H_{2'}), 7.58 (t, ³J = 6 Hz, 1H, H_{5''/6''}), 7.72 (t, ³J = 6 Hz, 1H, H_{5''/6''}), 7.90 (d, ³J = 6 Hz, 1H, H_{6'}), 8.00 (d, ³J = 6 Hz, 1H, H₇), 8.12 (d, ³J = 6 Hz, 1H, H_{5'}), 8.47 (s, 1H, H₄); ¹³C NMR (75 MHz, CDCl₃) δ : 55.5 (C_{4''}), 106.8 (C₃), 111.7 (C≡N), 113.9 (C_{3'}), 115.2 (C₈), 116.3 (C₁₀), 121.0 (C_{5'}), 125.4 (C_{2'}), 126.4 (C_{6''}), 128.8 (C_{5''}), 129.1 (C₄), 129.1 (C₇), 130.3 (C_{6'}), 135.1 (C₅), 137.4 (C_{1'}), 139.4 (C₆), 143.7 (C₂), 153.8 (C₉), 157.2 (C_{4'}). HRMS, *m/z*: 349.0947 found (calculated for C₂₁H₁₄N₂O₂Na [M+Na]⁺ requires 349.0948). Anal. Calcd for C₂₁H₁₄N₂O₂: C, 77.29; H, 4.32; N, 8.58. Found C, 77.31; H, 4.33; N, 8.57.

Standard procedure for the synthesis of 2-N-phenylamino-4H-chromene-3-carbonitrile 6(a-d) by reduction of 2-N-phenylimino-2H-[1]-benzopyran-3-carbonitrile 5(a-d).

In a 50 ml round-bottomed flask, provided with a magnetic stirrer and condenser, containing a suspension of 2-phenylimino-2H-[1]-benzopyran-3-carbonitrile **5** (12 mmol.) in 12 ml of dry THF cooled at 0°C was added small portions of commercial sodium borohydride (227 mg, 6 mmol.). The resulting reaction mixture was stirred vigorously (500 rpm) at 0 °C during 45 minutes. After stirring, the mixture was poured in 10 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 (3 x 10 ml). Recrystallization of the desired 2-phenylamino-4H-[1]-chromene-3-carbonitrile **6** was realized from a mixture of deionized water/ethanol, then dried under high vacuum (10⁻² Torr) at 25°C for 1 hour and afforded **6** as a powder.

8-Methoxy-2-N-phenylamino-4H-chromene-3-carbonitrile (6a)

Yield = 81%. Yellowish powder, mp = 122-124°C. ; IR (cm⁻¹): ν = 2195 (C≡N), 3320 (NH); ¹H NMR (300 MHz, CDCl₃) δ : 3.64 (s, 2H, H₄), 3.89 (s, 3H, OCH₃), 6.61 (br s, 1H, NH), 6.78 (dd, ³J = 6 Hz, 2H, H_{3'}), 7.08 (m, 2H, H_{4'}, H₇), 7.34 (m, 4H, H_{5'}, H₆, H_{2'}). ¹³C NMR (75 MHz, CDCl₃) δ : 24.6 (C₄), 56.2 (OCH₃), 56.8 (C₃), 110.7 (C₇), 119.8 (C≡N), 119.9 (C_{2'}), 120.1 (C_{4'}), 120.6 (C₅), 123.4 (C₁₀), 125.0 (C₆), 129.1 (C_{3'}), 138.1 (C_{1'}), 139.1 (C₉), 148.1 (C₂), 157.8 (C₈). HRMS, *m/z*: 301.0948 found (calculated for C₁₇H₁₄N₂O₂Na [M+Na]⁺ requires 301.0947). Anal. Calcd for C₁₇H₁₄N₂O₂: C, 73.37; H, 5.07; N, 10.07. Found C, 73.35; H, 5.10; N, 10.09.

8-Methoxy-2-(4-methoxyphenylamino)-4H-chromene-3-carbonitrile (6b)

Yield = 89%. Yellowish powder, mp = 148-150°C.; IR (cm⁻¹): ν = 2191 (CN), 3232 (NH); ¹H NMR (300MHz, DMSO-*d*₆) δ : 3.54 (s, 2H, H₄), 3.77 (s, 3H, H_{4''}), 3.79 (s, 3H, OCH₃), 6.78 (d, ³J = 6 Hz, 1H, H₇), 6.86 (d, ³J = 9 Hz, 2H, H_{2'}), 6.97 (d, ³J = 6 Hz, 1H, H₅), 7.09 (t, ³J = 6 Hz, 1H, H₆), 7.15 (d, ³J = 9 Hz, 1H, H_{3'}), 9.16 (br s, 1H, NH). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 25.3 (C₄), 55.7 (C_{4''}), 56.5 (OCH₃), 56.9 (C₃), 111.6 (C₇), 114.4 (C_{3'}), 120.1 (C≡N), 120.3 (C₅), 121.9 (C₁₀), 122.2 (C_{2'}), 125.3 (C₆), 132.6 (C_{1'}), 139.2 (C₉), 148.1 (C₂), 155.5 (C₈), 157.9 (C_{4'}). HRMS, *m/z*: 331.1056 found (calculated for C₁₈H₁₆N₂O₃Na [M+Na]⁺ requires 331.1053). Anal. Calcd for C₁₈H₁₆N₂O₃: C, 70.12; H, 5.23; N, 9.09. Found C, 70.14; H, 5.22; N, 9.11.

2-N-Phenylamino-4H-benzof[h]chromene-3-carbonitrile (6c)

Yield = 84%. Yellowish powder, mp = 188-190°C. IR (cm⁻¹): ν = 2193 (C≡N), 3278 (NH); ¹H NMR (300MHz, CDCl₃) δ : 3.98 (s, 2H, H₄), 6.66 (br s, 1H, NH), 7.17 (m, 2H, H₈, H_{4'}),

7.23 (d, $^3J = 6$ Hz, 2H, H_{2'}), 7.40 (t, $^3J = 6$ Hz, 2H, H_{3'}), 7.53 (t, $^3J = 6$ Hz, 1H, H_{5''/6''}), 7.64 (t, $^3J = 6$ Hz, 1H, H_{5''/6''}), 7.78 (t, $^3J = 6$ Hz, 2H, H_{7'}, H_{6'}), 7.87 (d, $^3J = 6$ Hz, 1H, H_{5'}). ¹³C NMR (75 MHz, CDCl₃) δ: 22.5 (C₄), 57.66 (C₃), 111.7 (C₈), 116.8 (C₁₀), 120.3 (C≡N), 121.0 (C_{2'}), 122.7 (C₇), 124.1 (C_{5'}), 125.4 (C_{4'}), 127.4 (C_{6''}), 128.5 (C_{5''}), 128.9 (C_{6'}), 130.9 (C₅), 131.1 (C₆), 137.7 (C_{1'}), 146.4 (C₂), 157.2 (C₉). HRMS, *m/z*: 321.0998 found (calculated for C₂₀H₁₄N₂O₂Na [M+Na]⁺ requires 321.09983). Anal. Calcd for C₂₀H₁₄N₂O: C, 80.52; H, 4.73; N, 9.39. Found C, 80.56; H, 4.71; N, 9.38.

2-(4-Methoxyphenylamino)-4H-benzo[h]chromene-3-carbonitrile (6d)

Yield = 85%. Yellowish powder, mp = 200-202°C; IR (cm⁻¹): ν = 2189 (C≡N), 3235 (NH); ¹H NMR (300MHz, CDCl₃) δ: 3.86 (s, 3H, H_{4''}), 3.96 (s, 2H, C₄), 6.51 (br s, 1H, NH), 6.94 (d, $^3J = 6$ Hz, 2H, H_{2'}), 7.11 (d, $^3J = 6$ Hz, 2H, H₈), 7.18 (t, $^3J = 6$ Hz, 2H, H_{3'}), 7.52 (t, $^3J = 6$ Hz, 1H, H_{5''/6''}), 7.63 (t, $^3J = 6$ Hz, 1H, H_{5''/6''}), 7.77 (dd, $^3J = 6$ Hz, 2H, H_{7'}, H_{6'}), 7.86 (d, $^3J = 6$ Hz, 1H, H_{5'}); ¹³C NMR (75 MHz, CDCl₃) δ: 22.4 (C₄), 55.5 (C_{4''}), 111.9 (C₃), 114.4 (C_{3'}), 116.8 (C₈), 120.6 (C₁₀), 122.7 (C_N), 124.1 (C_{2'}), 125.3 (C₇), 127.3 (C_{5'}), 128.5 (C_{6''}), 128.8 (C_{5''}), 130.3 (C_{6'}), 130.9 (C₅), 131.2 (C₆), 146.4 (C₂), 156.9 (C₉), 157.8 (C_{4'}). HRMS, *m/z*: 351.1105 found (calculated for C₂₁H₁₆N₂O₂Na [M+Na]⁺ requires 351.1104). Anal. Calcd for C₂₁H₁₆N₂O₂: C, 76.81; H, 4.91; N, 8.53. Found C, 76.79; H, 4.90; N, 8.52.

4.2 Biochemistry

4.2.1. Protein kinase preparations and assays

Kinase activities were assayed in:

- in buffer (A): 10 mM MgCl₂, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 μg/ml heparin
- or in buffer (B): 60 mM β-glycerophosphate, 30 mM p-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM MgCl₂, 1 mM DTT, 0.1 mM sodium orthovanadate
- or in buffer (C): MOPS 25 mM pH 7.5, 10 mM MgCl₂

with their corresponding substrate in the presence of 15 μM [γ-³³P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 μl following the assay described in [31]. Controls were performed with appropriate dilutions of dimethylsulfoxid. Full-length kinases are used unless specified. Peptide substrates were obtained from Proteogenix (Oberhausbergen, France)

- HsCDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 μg/μl of histone H1 as substrate;
- HsCDK5/p25 (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 μg/μl of histone H1 as substrate;
- SscCK1ε (casein kinase 1ε, porcine brain, native, affinity purified) was assayed in buffer B, with 0.022 μg/μl of the following peptide: RRKHA AIGSpAYSITA as CK1-specific substrate;
- SscGSK-3β (glycogen synthase kinase-3, porcine brain, native, affinity purified) was assayed in buffer A (+ 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);

- *HsHaspin-kd* (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;
- *HsPIM1* (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer B with 0.8 µg/µl of histone H1 (Sigma #H5505) as substrate;
- *MmCLK1* (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A (+ 0.15 mg/ml of BSA + 0.23 mg/ml of DTT) with 0.027µg/µl of the following peptide: GRSRSRSRSRSR as substrate.
- *RnDYRK1A-kd* (*Rattus norvegicus*, amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A (+0.5 mg/ml of BSA + 0.23 mg/ml of DTT) with 0.033 µg/µl of the following peptide: KKISGRLSPIMTEQ as substrate.

4.2.2. Cell culture and survival assays

Huh-7D12 (differential hepatocellular carcinoma, Ref ECACC: 01042712), Caco2 (differentiated colorectal adenocarcinoma, Ref ECACC: 86010202), MDA-MB-231 (breast carcinoma, Ref ECACC: 92020424), HCT-116 (actively proliferating colorectal adenocarcinoma, Ref ECACC: 91091005), PC3 (prostate carcinoma, Ref ECACC: 90112714), NCI-H727 (lung carcinoma, Ref ECACC: 94060303) cell lines were obtained from the ECACC collection and HaCaT (keratinocyte from Cell Lines Service, Eppelheim, Germany). Cells were grown according to ECACC recommendations [32]. The toxicity test of the compounds on these cells was as follows: 2 x 10³ cells for HCT-116 cells or 4 x 10³ for the other cells were seeded in 96 multi well plates in triplicate and left for 24 h for attachment, spreading and growing. Then, cells were exposed for 48 h to increasing concentrations of the compounds, ranging from 0.1 to 25 µM in a final volume of 120 µl of culture medium. Cells were fixed in cooled ethanol-acetic acid solution (95:5), nuclei were stained with Hoechst 3342 (Sigma) and counted using automated imaging analysis (Cellomics Arrayscan VTI/HCS Reader, Thermo/Scientific).

5. Acknowledgments

One of us (A.B.) 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 "Cancéropôle Grand Ouest" in "Molécules Marines, Métabolisme et Cancer" network, is gratefully acknowledged. The authors are grateful to the assistance of the staff of the CRMPO analytical chemistry core facility for HRMS and centesimal analysis (CRMPO platform ScanMAT UMS 2001 CNRS, Université de Rennes 1, Bat. 11A, Campus de Beaulieu, Rennes, France).

6. Conflicts of Interest: "The authors declare no conflict of interest."

7. References

- [1] Patil, S.A.; Patil, R.; Pfeffer, L.M.; Miller, D.D. (2013) Chromenes: potential new chemotherapeutic agents for cancer. *Future Med. Chem.* **14**, 1647-60. doi: 10.4155/fmc.13.126.
- [2] Cai, S.X.; Drewe, J.A.; Kasibhatla, S.; Kemnitzer, W.E.; Tseng, B.Y.; Blais, C.; Gourdeau, H.; Labrecque, D. (2008) Substituted 4-aryl-chromene as caspase activator and apoptosis inducer, and as antivasular agent for treatment of diseases due to

- vasculature overgrowth, such as solid tumors and ocular neovascularization. patent WO2008/005572 A2, 6 July 2006.
- [3] Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Zhao, J.; Crogan-Grundy, C.; Xu, L.; Lamothe, S.; Gourdeau, H.; Denis, R.; Ben, T.; Shailaja, K., Cai, S.X. (2007) Discovery of 4-aryl-4*H*-chromenes as a new series of apoptosis inducers using a cell- and caspase-based high-throughput screening assay. 3. Structure-activity relationships of fused rings at the 7,8-positions. *J. Med. Chem.* **50**, 2858-2864. doi:10.1021/jm070216c
- [4] Kemnitzer, W.; Shailaja, K.; Songchun, J.; Zhang, Z.; Zhao, J.; Jia, S.; Xu, L.; Crogan-Grundy, C.; Denis, R.; Barriault, N.; Vaillancourt, L.; Charron, S.; Dodd, J.; Attardo, G.; Labrecque, D.; Lamothe, S.; Gourdeau, H.; Tseng, B.; Drewe, J.; Cai, S.X. (2005) 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. Lett.* **15**, 4745-4751. doi:10.1016/j.bmcl.2005.07.066
- [5] Gulley J.M.D. (2010) A Phase I/II Trial of Crolibulin (EPC2407) Plus Cisplatin in Adults With Solid Tumors With a Focus on Anaplastic Thyroid Cancer (ATC) in <http://www.clinicaltrials.gov>, Identifier: NTC01240590.
- [6] Kasibhatla, S.; Gourdeau, H.; Meerovitch, K.; Drewe, J.; Reddy, S.; Qiu, L.; Zhang, H.; Bergeron, F.; Bouffard, D.; Yang, Q.; Herich, J.; Lamothe, S.; Cai, S.X.; Tseng, B. (2004) Discovery and mechanism of action of a novel series of apoptosis inducers with potential vascular targeting activity. *Mol. Cancer Ther.* **11**, 1365-74.
- [7] Gourdeau, H.; Leblond, L.; Hamelin, B.; Desputeau, C.; Dong, K.; Kianicka, I.; Custeau, D.; Boudreau, C.; Geerts, L.; Cai, S.X.; Drewe, J.; Labrecque, D.; Kasibhatla, S.; Tseng, B. (2004) Antivascular and antitumor evaluation of 2-amino-4-(3-bromo-4,5-dimethoxy-phenyl)-3-cyano-4*H*-chromenes, a novel series of anticancer agents. *Mol. Cancer Ther.* **11**, 1375-84.
- [8] Manero, F.; Gautier, F.; Gallenne, T.; Cauquil, N.; Grée, D.; Cartron, P-F.; Geneste, O.; René Grée, R.; Vallette, F.M.; Juin, P. (2006) 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**, 2757-2764. doi:10.1158/0008-5472.CAN-05-2097
- [9] Doshi, J.M.; Tian, D.; Xing, C. (2006) 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. *J. Med. Chem.* **49**, 7731-7739. doi: 10.1021/jm060968r
- [10] Grée, D.; Vorin, S.; Manthati, V.L.; Caijo, F.; Guillaume Viault, G.; Manero, F.; Juin, P.; Grée, R. (2008) The synthesis of new, selected analogues of the pro-apoptotic and anticancer molecule HA 14-1. *Tetrahedron Lett.* **49**, 3276-3278. doi: 10.1016/j.tetlet.2008.03.070
- [11] Wang, J-L.; Liu, D.; Zhang, Z-J.; Shan, S.; Han, X.; Srinivasula, S.M.; Croce, C.M.; Alnemri, E.S.; Huang, Z. (2000) Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *PNAS* **97**, 7124-7129.
- [12] Weyland, M.; Manero, F.; Paillard, A.; Grée, D.; Viault, G.; Jarnet, D.; Menei, P.; Juin, P.; Chourpa, I.; Benoit, J-P.; Grée, R.; Garcion, E. (2011) Mitochondrial targeting by use of lipid nanocapsules loaded with SV30, an analogue of the small-molecule Bcl-2 inhibitor HA 14-1. *J. Control. Rel.* **151**, 74-82. doi: 10.1016/j.conrel.2010.11.032
- [13] Wood, D.L.; Panda, D.; Wiernicki, T.R.; Wilson, L.; Jordan, M.A.; Singh, J.P. (1997) Inhibition of mitosis and microtubule function through direct tubulin binding by a novel antiproliferative naphthopyran LY290181. *Mol. Pharm.* **52**, 437-444.

- [14] Panda, D.; Singh, J.P.; Wilson, L. (1997) Suppression of microtubule dynamics by LY290181. *J. Biol. Chem.* **272**, 7681-7687.
- [15] Sciabola, S.; Carosati, E.; Cucurull-Sanchez, L.; Baronc, M.; Mannhold, R. (2007) Novel TOPP descriptors in 3D-QSAR analysis of apoptosis inducing 4-aryl-4H-chromenes: Comparison versus other 2D- and 3D-descriptors. *Biorg. Med. Chem.* **15**, 6450-6462. doi:10.1016/j.bmc.2007.06.051
- [16] Souchet, B.; Audrain, M.; Billard, J-M; Dairou, J.; Fol, R.; Orefice, N.; Tada, S.; Dufayet, G. Alves, S.; Potier, B.; Dutar, P.; Limanton, E.; Carreaux, F.; Bazureau, J-P.; Meijer, L.; Janelle, N.; Braudeau, J.; Cartier, N. (2019) Inhibition of DYRK1A proteolysis modifies its kinase specificity and rescues Alzheimer phenotype in APP/PS1 mice. *Acta Neuropathol. Commun.* **7**, article: 46(2019). <https://doi.org/10.1186/s40478-019-0678-6>
- [17] Nguyen, T.L.; Duchon, A.; Manousopoulou, A.; Loaëc, N.; Villiers, B.; Pani, G.; Harsan, L.; Limanton, E.; Bazureau, J-P.; Carreaux, F.; Garbis, S.D.; Meijer, L.; Herault, Y. (2018) Correction of cognitive deficits in mouse models of Down syndrome by pharmacological inhibitor of DYRK1A. *Disease Models & Mechanisms* **11**, dmm035634. doi: 10.1242/dmm.035634
- [18] Dago, C.D.; Le Maux, P.; Roisnel, T.; Brigaudeau, C.; Bekro, Y-A.; Mignen, O.; Bazureau, J-P. (2018) Preliminary structure-activity relationship (SAR) of a novel series of pyrazole SKF-96365 analogues as potential Store-Operated Calcium Entry (SOCE) inhibitors. *Int. J. Mol. Sci.* **2018**, *19*, 856-879. doi:10.3390/ijms19030856.
- [19] Bouattour, A.; Fakhfakh, M.; Abid, S.; Paquin, L.; Le Guével, R.; Corlu, A.; Ruchaud, S.; Bach, S.; Ammar, H.; Bazureau, J-P. (2017) Microwave assisted a practical synthesis of 4-imino-3-phenyl-3,4-dihydro-1H-chromeno[2,3-d]pyrimidine-2(5H)-thione derivatives and exploration of their biological activities. *Arkivoc*, **iv**, 291-302. doi: <http://dx.doi.org/10.3998/ark.5550190.p010.040>
- [20] Bouattour, A.; Fakhfakh, M.; Abid, S.; Paquin, L.; Ammar, H.; Bazureau, J-P. (2017) Microwave mediated efficient syntheses of N-3 substituted 9-methoxy-4H-[1]-benzopyrano[2,3-d]pyrimidine-4(5H)-imine and formamidine derivatives from methyl 3-cyano-8-methoxy-4H-[1]-benzopyran-2-yl methanimidate. *Synthesis*, 3768-3774. doi: 10.1055/s-0036-1588825
- [21] Fakhfakh, M.; Turki, H.; Fery-Forgues, S.; El Gharbi, R. (2010) The synthesis and optical properties of novel fluorescent iminocoumarins and bis-iminocoumarins: Investigations in the series of urea derivatives. *Dyes & Pigments* **84**,108-113. doi:10.1016/j.dyepig.2009.07.003
- [22] Ben Maktouf, L.; Kammoun, M.; Ammar, H.; Abid, S. (2011) Réactivité des 3-cyano iminocoumarines vis-à-vis du phénylsemicarbazide. *J. Soc. Chem. Tunis.* **13**, 101-106. <http://www.sctunisie.org/journal-sct/index.php?categorie15/volume-13-dec-2011>
- [23] Benhaoua, C.; Kasmi, S.; Rahmouni, M.; Bazureau, J-P. (2018) Synthesis of some enamionone derivatives under solvent-free process. *Maroccan J. Het. Chem.* **17**, 50-60.
- [24] Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J.A.; Snyder, G.L.; Greengard, P.; Biernat, J.; Mandelkow, E.M.; Eisenbrand, G.; Meijer, L. (2001). Indirubins inhibit glycogen synthase kinase-3 β 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**, 251-260. doi: 10.1074/jbc.M002466200
- [25] Sonawane, Y.A.; Taylor, M.A.; Napoleon, J.V.; Rana, S.; Contreras, J.I.; Natarajan, A. (2016) Cyclin dependent kinase 9 inhibitors for cancer therapy. *J. Med. Chem.* **59**, 8667-8684. doi: 10.1021/acs.jmedchem.6b00150.

- [26] Rena, G.; Bain, J.; Elliott, M.; Cohen, P. (2004) D4476, a cell-permeant inhibitor of CK1, suppresses the site-specific phosphorylation and nuclear exclusion of FOXO1a. *EMBO Rep* **5**, 60-65. doi: 10.1038/sj.embor.7400048
- [27] Primot, A.; Baratte, B.; Gompel, M.; Borgne, A.; Liabeuf, S.; Romette, J.L.; Costantini, F.; Meijer, L. (2000) Purification of GSK-3 by Affinity Chromatography on Immobilized Axin. *Protein Exp. Purif.* **20**, 394-404. doi:10.1006/prep.2000.1321
- [28] Blanco-Aparicio, C.; Carnero, A. (2013) Pim kinases in cancer: diagnostic, prognostic and treatment opportunities. *Biochem. Pharm.* **85**, 629-643. doi: 10.1016/j.bcp.2012.09.018
- [29] Karthikeyan, J.P.; Moorthy, N.S.; Waiker, D.K.; Jain, A.K.; TRivedi, P. (2014) Human CDC2-like kinase 1 (CLK1): a novel target for Alzheimer's disease. *Curr. Drug Targets* **15**, 539-550. doi:10.2174/1389450115666140226112321
- [30] Fant, X.; Durieu, E.; Chicanne, G.; Payraastre, B.; Sbrissa, D.; Shisheva, A.; Limanton, E.; Carreaux, F.; Bazureau, J-P.; Meijer, L. (2014) CLK/DYRK kinases inhibitor Leucettine L41 induces mTOR-dependent autophagy. Implication for Alzheimers' disease. *Mol. Pharm.* **85**, 441-450. doi: <https://doi.org/10.1124/mol.113.090837>
- [31] Bach, S.; Knockaert, M.; Reinhardt, J.; Lozach, O.; Schmitt, S.; Baratte, B.; Koken, M.; Coburn, S.P.; Tang, L.; Jiang, T.; Liang, D.C.; Galons, H.; Dierick, J-F.; Pinna, L.A.; Meggio, F.; Totzke, F.; Schachtele, C.; Lerman, A.S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. (2005) Roscovitine targets, protein kinases and pyridoxal kinase. *J Biol Chem* **280**, 31208-31219. doi: 10.1074/jbc.M500806200
- [32] Nakabayashi, H.; Taketssa, K.; Miyano, K.; Yamane, T.; Sato, J. (1982) Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. *Cancer Res.* **42**, 3858-3863