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N-benzyl derivatives of 2-amino-8-methoxy-4*H*-chromene-3-carbonitrile: synthesis *via* reductive amination, *in silico* ADME profiling & exploration of their effects against protein kinases.

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pharmaceuticals MDPI

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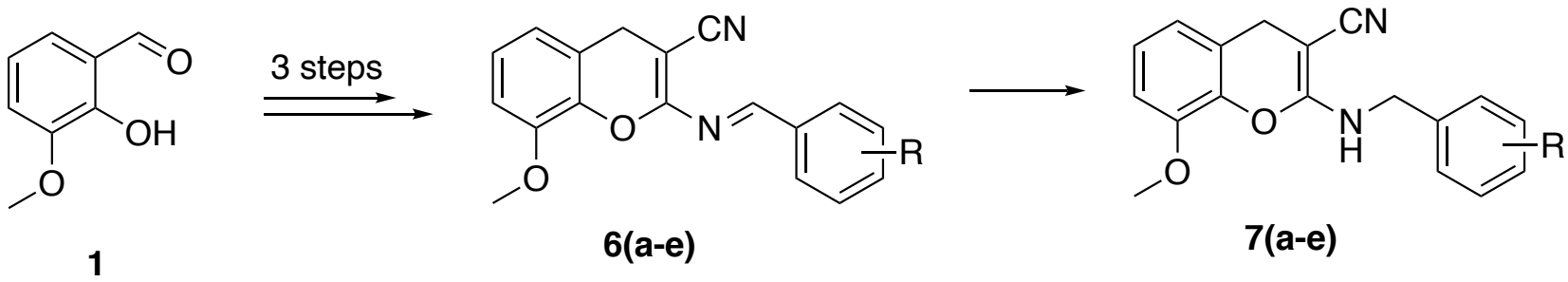
N-benzyl derivatives of 2-amino-8-methoxy-4H-chromene-3-carbonitrile: synthesis *via* reductive amination, *in silico* ADME profiling & exploration of their effects against protein kinases.

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

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.

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

Introduction

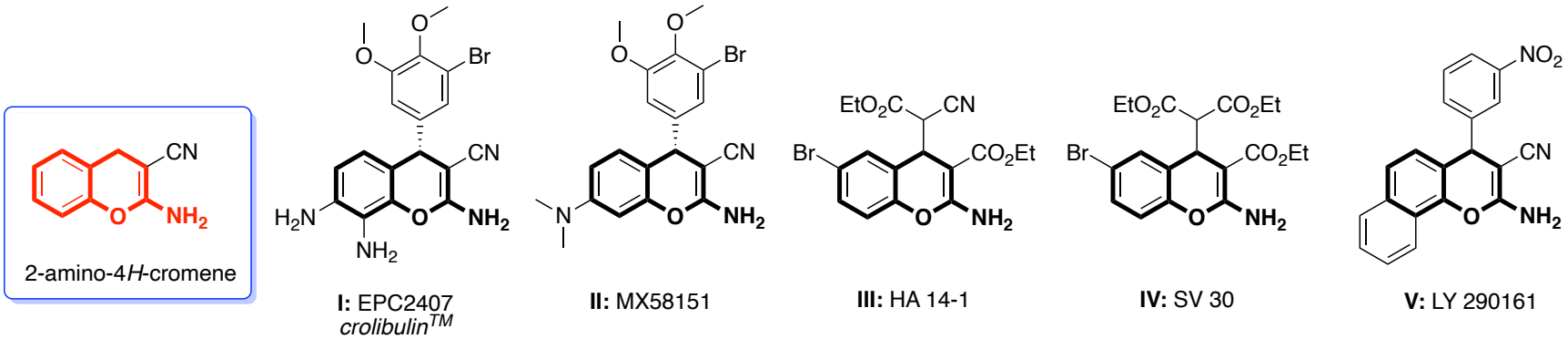


Figure 1: Bioactive 2-amino-4*H*-chromene derivatives reported in literature

References for:

- **EPC2407**: Cai et al., WO 2008/005572 A2, 6 July 2006
- **MX58151**: Kasibhatla et al. Mol Cancer Ther 11 (2004) 1365-1374
- **HA14-1**: Manero et al. Cancer Res 66 (2006) 2757-2764
- **SV30**: Weyland et al. J Control Release 151 (2011) 74-82
- **LY 290 161**: Wood et al. Mol Pharm 52 (1997) 437-444

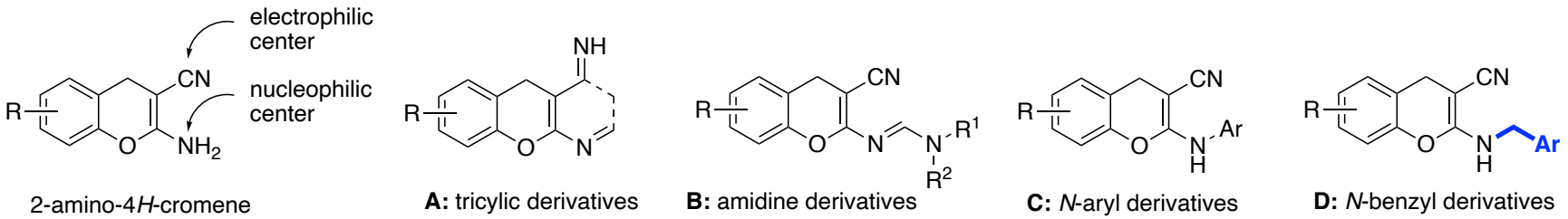


Figure 2: Possibilities of molecular diversity offered by the 2-amino-4*H*-chromene-3-carbonitrile platform developed in our laboratory

References for:

- **A**: Bouattour et al. Arkivoc 4 (2017) 291-302
- **B:** Bouattour et al. Synthesis 49 (2017) 3768-3774
- **C:** Bouattour et al. Int. J. Org Chem 10 (2020) 88-103

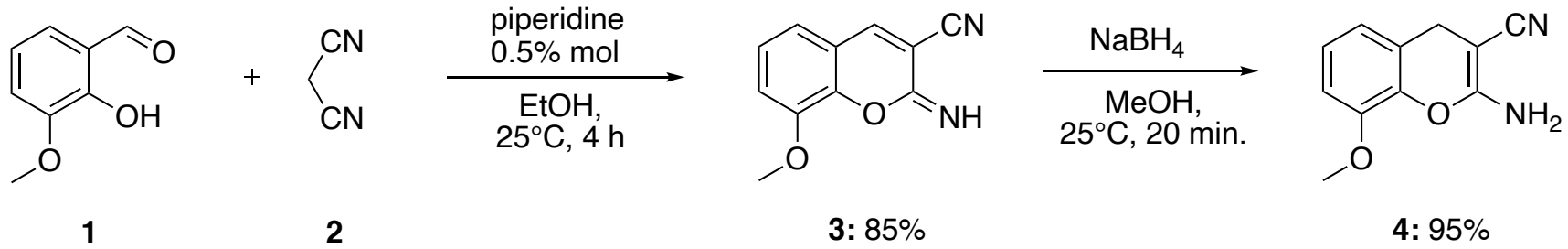
Our goals:

1. To prepare directly compounds **D** from « 2-amino-4*H*-chromene platform » with halogenated derivatives
2. To explore their effects on protein kinases (PKs) in a first approach.

Results and discussion

1. Chemistry section

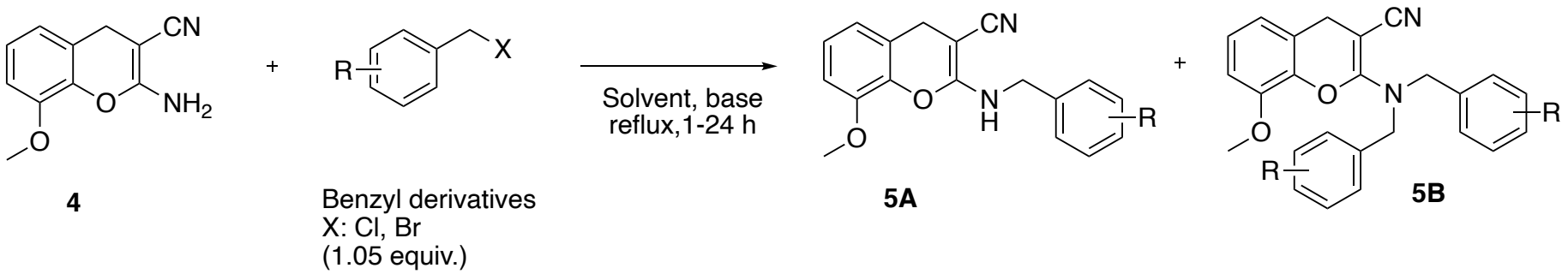
1.1 Preparation of the 2-amino-4*H*-chromene platform (**4**)



1 **2** **3:** 85% **4:** 95%

Compound (**4**) prepared in 2 steps with 81% yield

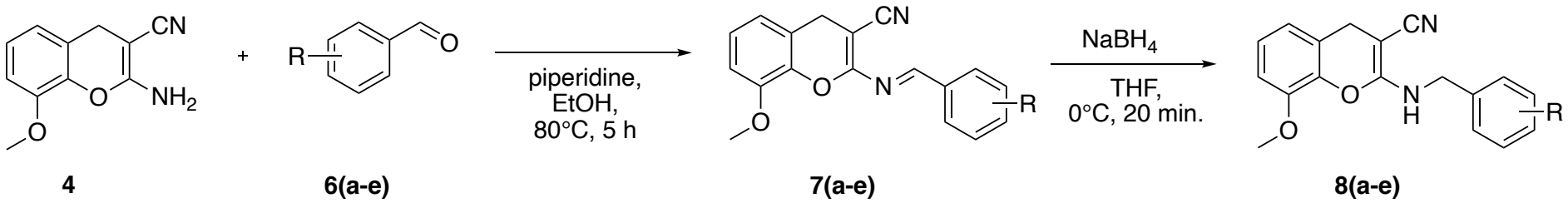
1.2 Direct *N*-alkylation of 2-amino-4H-chromene (**4**) with halogeno benzyl derivatives



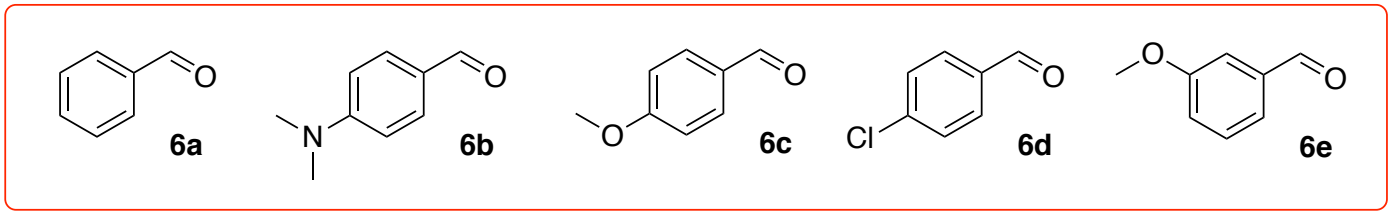
Analysis of the crude reaction mixture by ^{1}H NMR in DMSO-d_6 solution: Difficulties to control the monoalkylation of (**4**) to obtain exclusively (**5A**) whatever the reaction conditions used (solvent of reaction, reflux duration, mineral or organic base)

A possible solution to built the expected compound (**5A**) is the reductive amination ?

1.3 Preparation of 2-benzylamino-4*H*-chromene-3-carbonitriles **8(a-e)** by reductive amination

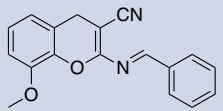
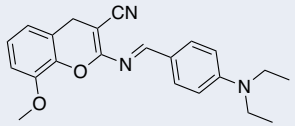
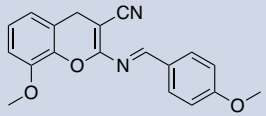
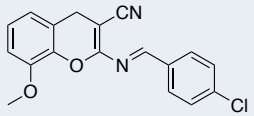
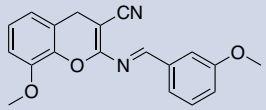


4 **6(a-e)** **7(a-e)** **8(a-e)**



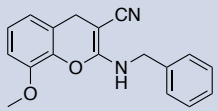
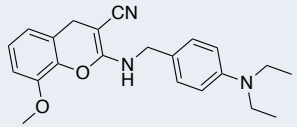
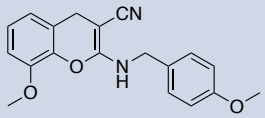
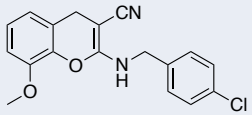
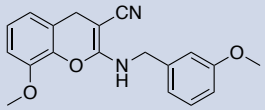
Step 1: the use of piperidine increase the kinetic of the condensation, **4** + **6** -> **7**
Step 2: classical reaction conditions for reduction of imine function of **7**

Table 1: Results for th preparation of 2-[(*E*)-(benzylidene)amino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a-e)**

Structure of compound 7		Yield of 7 (%) [a]	Overall yield (%) [b]	δ CH= of 7 (ppm) [c]	
7a		70	45	8.88 [d]	162.4 [d]
7b		52	33	8.81	160.8
7c		90	57	8.88	160.9
7d		80	51	8.89	160.2
7e		78	50	8.92	161.7

[a] Yield of isolated product **7**. [b] Overall yield of **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.

Table 2: Results for th preparation of 2-benzylamino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a-e)**

Structure of compounds 8		Yield of 8 (%) [a]	Overall yield (%) [b]	δ CH_2Ar of 8 (ppm) [c]	ν NH of 8 (cm^{-1})
8a		96	43	4.38 [d]	3325
8b		96	32	4.48	3337
8c		97	56	4.51	3332
8d		80	41	4.51	3318
8e		78	39	4.45	3362

[a] Yield of isolated product **8**. [b] Overall yield of **8** calculated from compound **3**. [c] ^{1}H NMR in DMSO-d_6 solution with TMS as internal reference. [d] ^{1}H NMR and ^{13}C NMR in $CDCl_3$ solution.

1.4 Physicochemical properties and *in silico* ADME studies

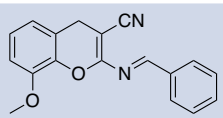
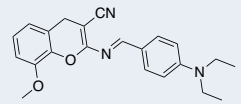
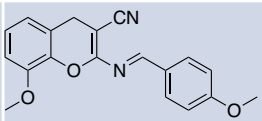
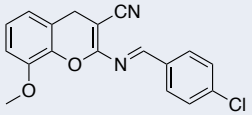
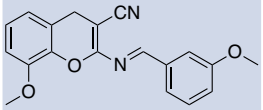
Lipinski Rule of Five (RO5)

Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors HBD (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds): HBD ≤ 5
- No more than 10 hydrogen bond acceptors HBA (all nitrogen or oxygen atoms): HBA ≤ 10
- A molecular mass MW less than 500 gr/mol.: MW ≤ 500
- An octanol-water partition coefficient (log $P_{o/w}$) that does not exceed 5: log $P_{o/w}$ ≤ 5
- Molar refractivity should be between 40-130: 40 < MR < 130

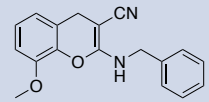
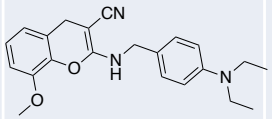
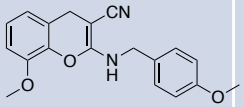
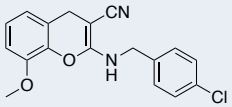
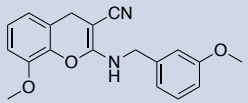
Reference:
Lipinski CA (December 2004). "Lead- and drug-like compounds: the rule-of-five revolution". *Drug Discovery Today: Technologies* **1 (4)**: 337-341. doi:10.1016/j.ddtec.2004.11.007

Table 3: Results of physicochemical properties for 2-[(*E*)-(benzylidene)amino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a-e)**.

Structure of 7		MW (g/mol)	Log P	Log S	tPSA (Å²)	HBA	HBD	RB	Fract. Csp³	LV
7a		290.32	3.26	-3.89	54.61	4	0	3	0.11	0
7b		361.44	3.95	-4.60	57.85	4	0	6	0.27	0
7c		320.34	3.27	-3.95	63.84	5	0	4	0.16	0
7d		324.76	3.80	-4.48	54.61	4	0	3	0.11	0
7e		320.34	3.27	-3.95	63.84	5	0	4	0.16	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.

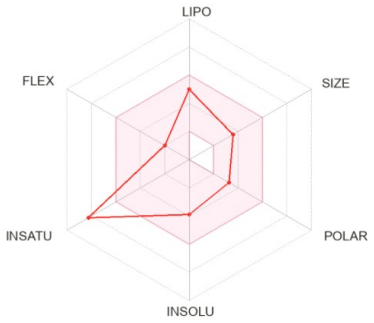
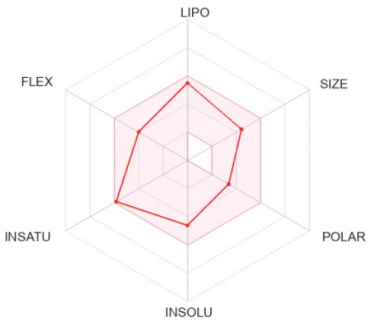
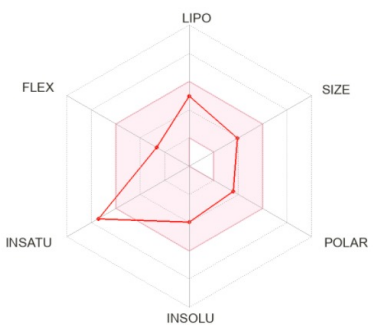
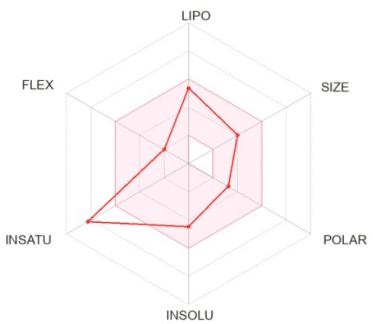
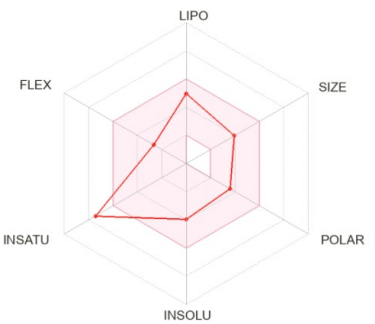
Table 4: Results of physicochemical properties for 2-benzylamino-8-methoxy-4*H*-chromene 3-carbonitrile **8(a-e)**

Structure of 8		MW (g/mol)	Log P	Log S	tPSA (Å²)	HBA	HBD	RB	Fract. Csp³	LV
8a		293.33	3.13	-4.29	54.28	3	1	4	0.17	0
8b		363.45	3.75	-5.00	57.62	3	1	7	0.32	0
8c		322.36	3.10	-4.36	63.51	4	1	5	0.21	0
8d		326.78	3.67	-4.89	54.28	3	1	4	0.17	0
8e		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.

Swiss ADME of *Swiss Institute of Bioinformatics*. Accessed 4 May 2022. http://www.swissadme.ch/

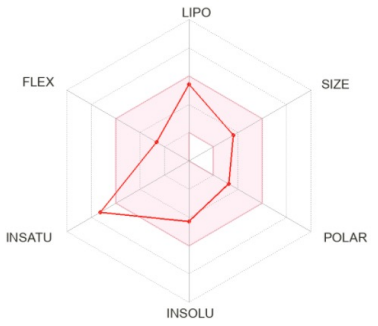
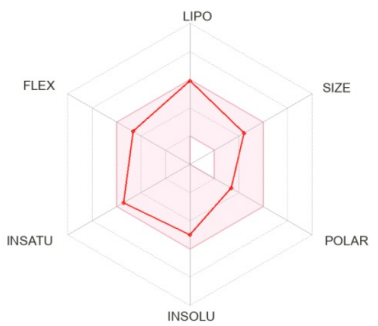
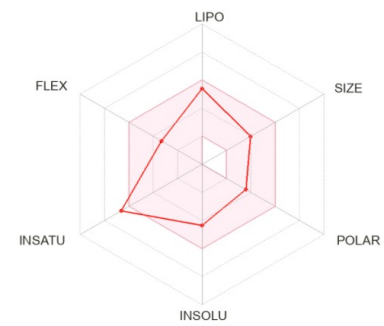
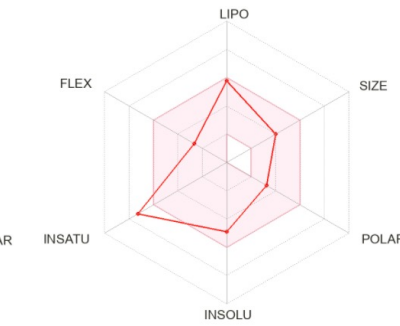
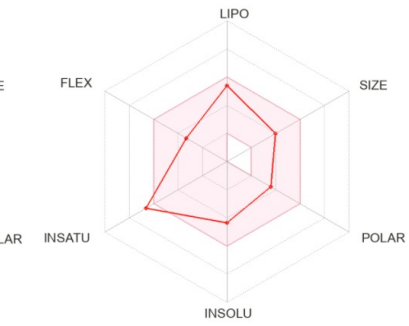
Table 5: Bioavailability radar related to the physicochemical properties of 2-[(*E*)-(benzylidene)amino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a-e)**.

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

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.

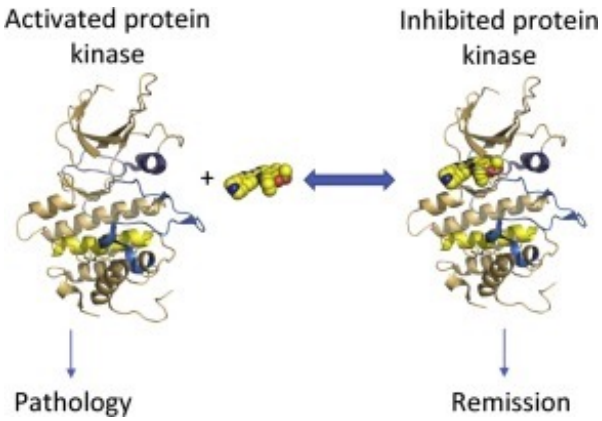
Table 6: Bioavailability radar related to the physicochemical properties of 2-benzylamino-8-methoxy-4*H*-chromene 3-carbonitrile **8(a-e)**.

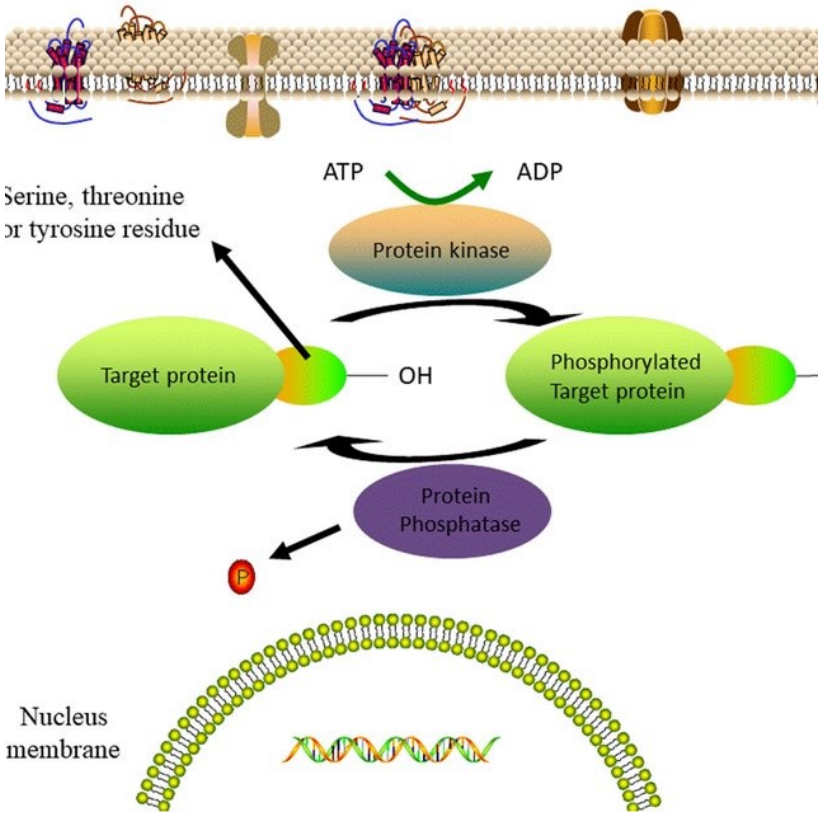
8a %Abs: 55	8b %Abs: 55	8c %Abs: 55	8d %Abs: 55	8e %Abs: 55
				

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.

Note: *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. Except for saturation, the radar plot shows whether all compounds **7** and **8** are in the pink area.*

2. Biology section: protein kinases (PKs)





The catalytic cycle for protein phosphorylation by a protein kinase.

Protein kinases inhibitors

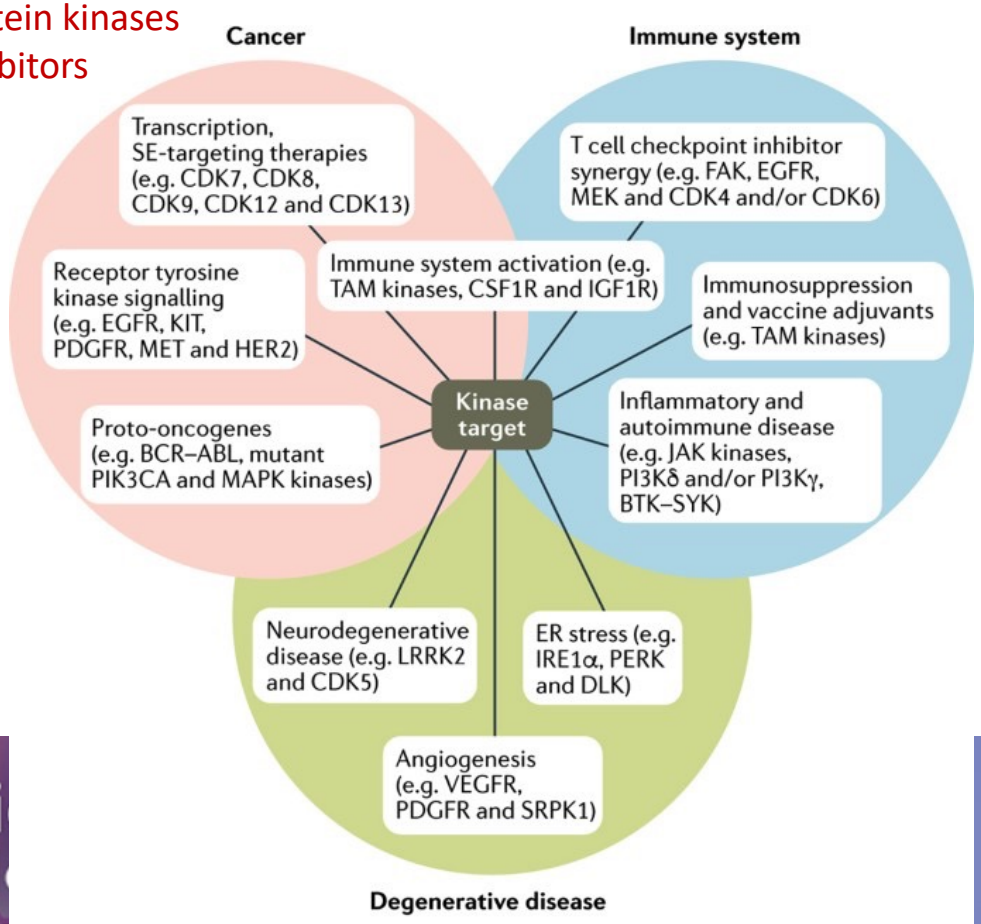


Table 7: Effects of 2-[(*E*)-(benzylidene)amino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a-e)** on the catalytic activity of six protein kinases (PKs).

Compound	Concent. (μM)	CDK5-p25	CDK9/ cyclin T	Pim 1	Haspin	*Ssc*GSK 3α/β	*Ssc*CK1δ/ε
7a	10	>100	77	26	79	83	63
7a	1	>100	104	99	98	77	>100
7b	10	>100	88	55	71	92	49
7b	1	99	93	52	80	89	75
7c	10	94	>100	>100	66	88	>100
7c	1	100	>100	>100	96	83	69
7d	10	97	79	60	68	65	72
7d	1	95	72	>100	>100	80	59
7e	10	>100	88	73	46	71	>100
7e	1	>100	83	88	69	73	>100

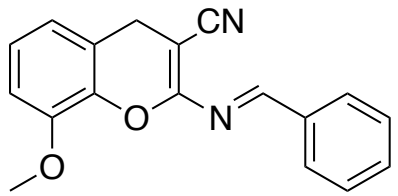
ATP concentration in the kinase assays was 15 μM/L. Results are expressed in % of maximal activity, *i.e.* measured in the absence of inhibitor but with an equivalent of dose of DMSO (solvent of the tested compounds) (values are means, n = 2). Kinases are from human origin except for it is specified *Ssc, Sus scrofa domesticus*).
NB: >100 indicate that the compound inhibit the enzymatic activity at the tested concentration.

Table 8: Effects of 2-benzylamino-8-methoxy-4*H*-chromene 3-carbonitrile **8(a-e)** on the catalytic activity of six protein kinases (PKs).

Compound	Concent. (μM)	CDK5-p25	CDK9/ cyclin T	Pim 1	Haspin	*Ssc*GSK 3α/β	*Ssc*CK1δ/ε
8a	10	84	>100	81	>100	>100	97
8a	1	>100	96	86	>100	100	97
8b	10	>100	85	100	>100	93	>100
8b	1	>100	>100	87	97	99	>100
8c	10	>100	88	73	99	84	>100
8c	1	>100	84	74	66	97	92
8d	10	97	89	68	71	91	86
8d	1	>100	88	>100	>100	>100	74
8e	10	>100	79	87	>100	75	59
8e	1	>100	84	76	59	75	83

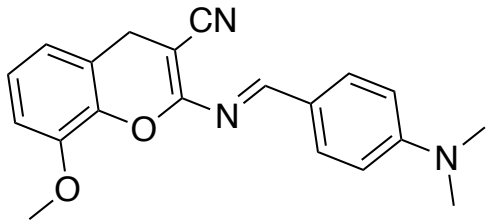
ATP concentration in the kinase assays was 15 μM/L. Results are expressed in % of maximal activity, *i.e.* measured in the absence of inhibitor but with an equivalent of dose of DMSO (solvent of the tested compounds) (values are means, n = 2). Kinases are from human origin except for it is specified *Ssc, Sus scrofa domesticus*).
NB: >100 indicate that the compound inhibit the enzymatic activity at the tested concentration.

Figure 3: 2-[(*E*)-(benzylidene)amino-8-methoxy-4*H*-chromene 3-carbonitrile **7(a,b)** and **7e** (with their 3D structure) which are bioactive against protein kinases Pim1 and *Hs*Haspin. *Hs, Homo sapiens*.



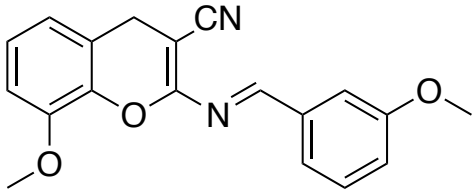
7a

Hs Pim1: 26% at 10 μM



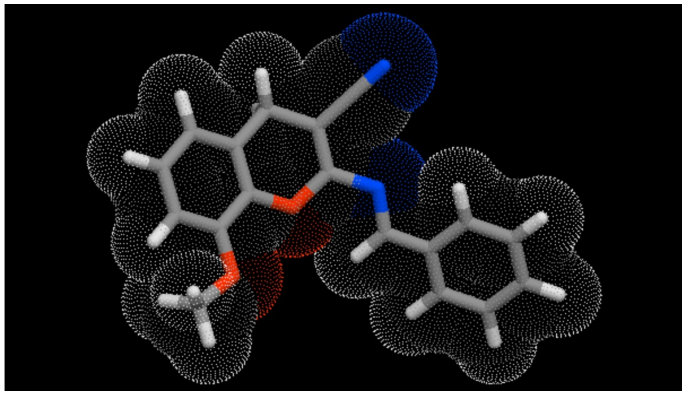
7b

Hs Pim1: 52% at 1 μM

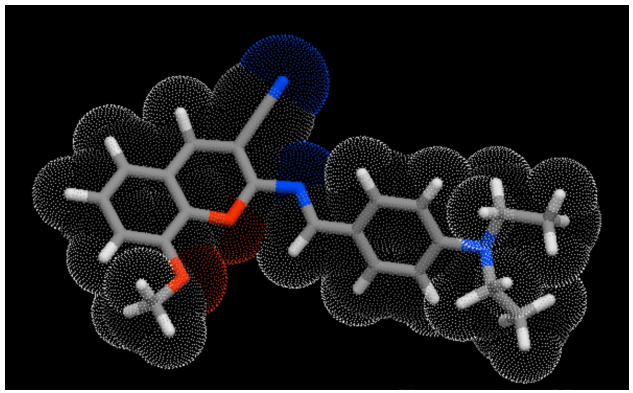


7e

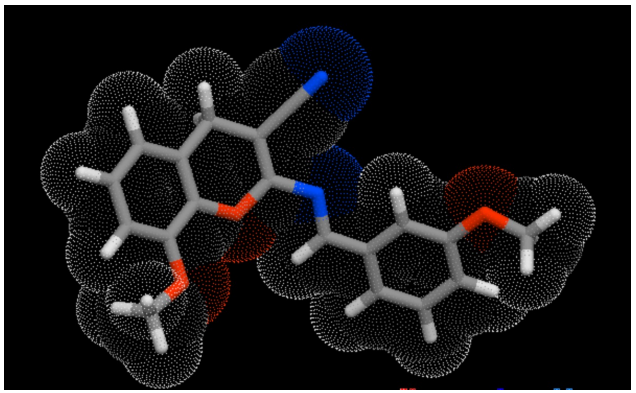
Hs Haspin: 46% at 10 μM



3D structure of **7a** (*)



3D structure of **7b** (*)



3D structure of **7e** (*)

(*) 3D structure generated from https://www.molinspiration.com/cgi-bin/galaxy

Conclusions

- A practical 4 steps approach to new *N*-benzylamino-8-methoxy-4*H*-chromene-3-carbonitriles **8(a-e)** was developed in moderate to good overall yields *via* reductive amination

- Reductive amination indirectly solves the difficulties of controlling the mono-alkylation of platform **4** with halogenated derivatives (derivatives of benzyl chloride and bromide as examples),

- The physicochemical properties of compounds **8(a-e)** and also their aldimine precursors **7(a-e)** have been determined using the Swiss ADME server plateform according to the Lipinski's descriptors.

- Biological assays with a panel of 6 protein kinases (as *Hs*CDK5-p25, *Hs*CDK9/Cyclin T, *Hs*Pim1, *Hs*Haspin, *Ssc*GSK3α/β and *Ssc*CK1δ/ε) showed that intermediates **7(a,b)** and **7e** are potentially interesting because they presented good % of residual activities against *Hs*Pim1 and *Hs*Haspin.

- **Near future:** a complete Relationship Struture-Activity (RSA) on *N*-benzylamino-8-methoxy-4*H*-chromene-3-carbonitriles **8** is under progress for identification of a better protein-kinase inhibitor.

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- **For financial support for people working at ISCR and UR1 (F)**

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