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Microwave-assisted practical synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives and exploration of their biological activities

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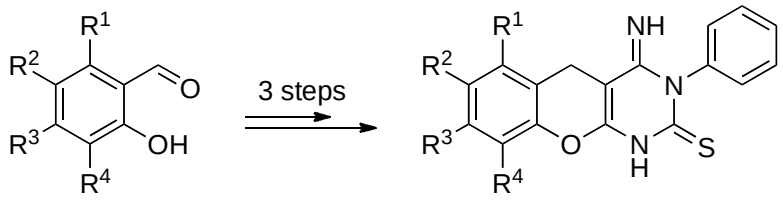
Abstract

The synthesis of a series of new 4-imino-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thiones **6(a-f)** without substituent in C-5 position using microwave dielectric heating is reported. These new compounds were obtained in three steps with good overall yields (21-50%).



1(a-f) **6(a-f)**

Keywords: Chromeno[2,3-*d*]pyrimidine, microwave, aminochromene, iminocoumarin, kinase inhibition, tumoral cell line

Introduction

The interest for 4*H*-chromenes and their derivatives increased since the two last decades because this class of compounds are components of many naturally occurring products and have also been subjected to structural modifications for potential medicinal properties. As examples (Figure 1), the racemic 15-(3-methoxyphenyl)-9,11,12,15-tetrahydro-10*H*,14*H*-benzo[5,6]chromeno[2,3-*d*]pyrido[1,2-*a*]pyrimidine-14-imine ***(I)***[1,2] was identified as novel inhibitor of acetylcholine esterase (*h*AChE IC_{50} 58 nM) and butyrylcholine esterase (*h*BuChe IC_{50} 302 nM) with a selectivity of 5.2. However, this compound presented very weak activity in $A\beta_{1-42}$ aggregation for Alzheimer's disease therapy. The (*R,S*) *N'*-(4-(4-chlorophenyl)-3-cyano-7-methoxy-4*H*-chromene-2-yl)-*N*-carbamoylformamimidamide ***(II)*** showed good *in vitro* cytotoxic activity on a human breast tumor cell line (MCF, IC_{50} 8 nM).[3] Based on a structure-activity relationship (SAR) and molecular docking studies on the 6*H*, 7*H*-chromeno[4,3-*b*]chromen-6-one scaffold, the addition of an aromatic ring in position C-7 ***(III)*** suggested that the resulting planar ring system could be an important scaffold to maintain anti-HIV activity against both wild type and multi-drug resistant HIV strains.[4]

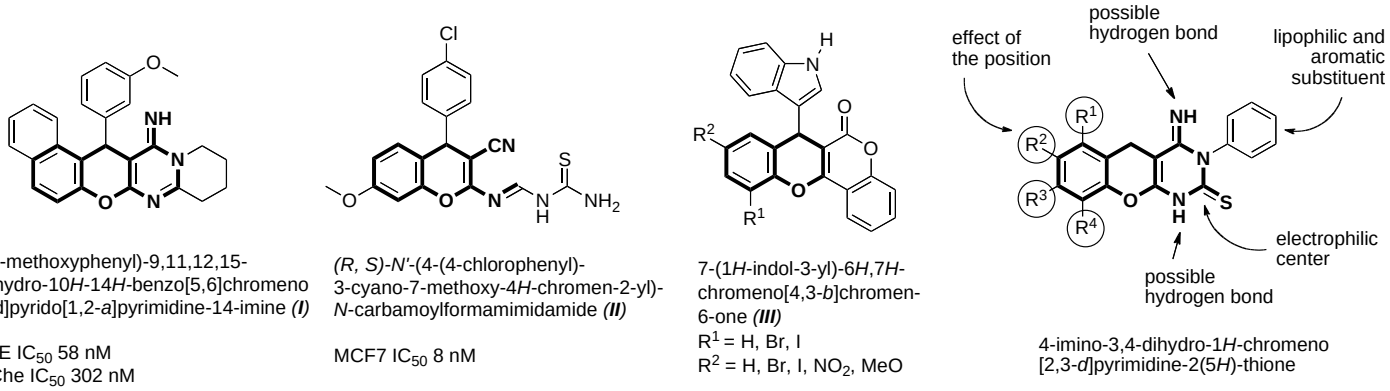


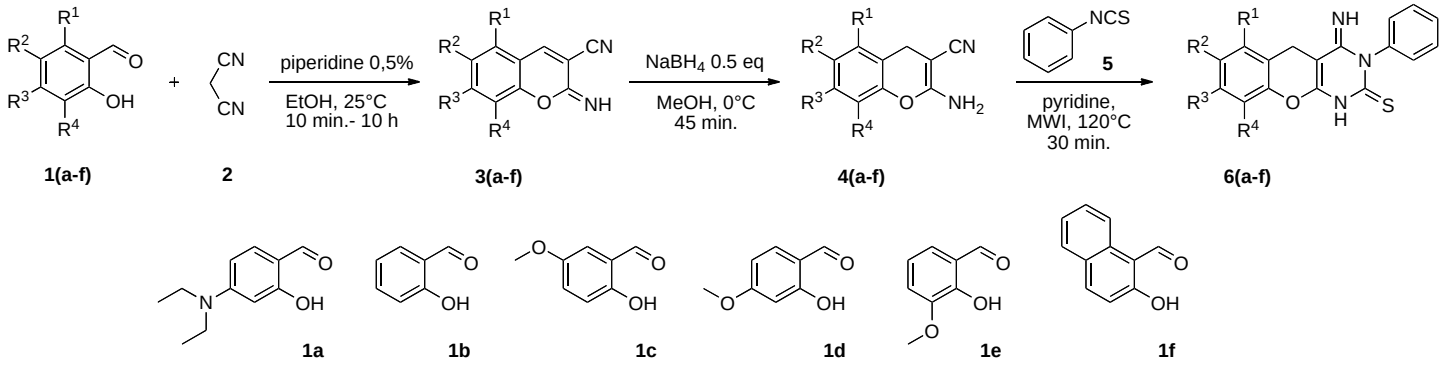
Figure 1. Examples of bioactive 3-aryl chromene derivatives and structure of the desired 4-imino-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives.

For the synthesis of 2-amino-4*H*-chromeno derivatives, a literature survey showed that there are two major methods of construction. The first involved reaction of benzylidenemalonitrile derivatives with various phenols, naphtols under basic conditions,[5] which gave 2-amino-4*H*-chromene-3-carbonitrile substituted in position C-4 by a phenyl group.[6] In addition, a three component approach from various aromatic aldehydes, malonitrile and α-naphtols was realized in PEG-400 at 100 °C during 2-3 hours without any catalyst,[7] which produced 2-amino-4-phenyl-4*H*-benzo[*h*]chromeno-3-carbonitrile. The second is based on heterocyclization of *O*-acetyl salicylic acid derivative with malonitrile in aqueous NaOH solution followed by subsequent acidification, that afforded the corresponding substituted 2-amino-4-oxo-4*H*-chromene-3-carbonitriles.[8,9] It is noteworthy that the antitubercular (*Mycobacterium tuberculosis*) and antimicrobial (Gram-positive and Gram-negative bacterial species) activities of these compounds have been investigated.

In this context, we decided to develop a series of 2-amino-4*H*-[1]chromene-3-carbonitrile without substituent in C-4 position *via* the 3-cyanoiminocoumarin route.[10,11] For this project, we studied also the chemical reactivity of the 2-amino group of chromene-3-carbonitrile with aryl isothiocyanate under microwave dielectric heating in order to produce novel 4-imino-3,4-dihydro-1*H*-chromene[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives (Figure 1) and to evaluate their impact respectively on protein kinase activity and antiproliferative activity with representative tumoral cell lines for possible detection of biological properties.

Results and Discussion

Access to the planned 4-imino-3,4-dihydro-1*H*-chromene[2,3-*d*]pyrimidine-2(5*H*)-thiones **6** and their 2-amino-4*H*-1-chromene-3-carbonitrile precursors **4** is outlined in Scheme 1. Owing to that our synthetic strategy involved the preparation of iminocoumarin-3-carbonitriles **3** as starting materials, we selected a series of aromatic aldehydes **1(a-f)** bearing diethylamino- or methoxy- groups at various positions for the introduction of molecular diversity on the expected compounds. The desired iminocoumarins **3(a-f)** or 2-imino-2*H*-[1]-benzopyran-3-carbonitriles were easily prepared by classical heterocyclization from an equimolecular mixture of substituted 2-hydroxybenzaldehyde **1** and malonitrile **2** in the presence of 0.5% piperidine in EtOH according to a procedure developed in our laboratory.[12] After a reaction time varying from 10 min. to 10 hours and elimination of volatile compound *in vacuo*, the desired compounds **3(a-f)** were obtained in yields ranging from 60 to 96% (Table 1).



Scheme 1. Synthesis of 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** and 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** under microwave irradiation.

For the second step, transformation of the 2-imino-2*H*-[1]-benzopyran-3-carbonitrile **3(a-f)** into 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** could be readily accomplished in good yields (50 to 90%) using 0.5 equivalent of $NaBH_4$ in MeOH at 0 °C during 45 min. Separation of the desired compound **4** from the crude reaction mixture was easily realized by addition of deionized water and the resulting precipitated product **4** was collected by simple filtration. With the 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** in hand, we decided to explore the addition of phenylisothiocyanate **5** to **4** under microwave irradiation[13,14] for the preparation of 4-imino-3,4-dihydro-1*H*-chromene[2,3-*d*]pyrimidine-2(5*H*)-thiones **6**. The underlying idea for the use of microwave dielectric heating in organic synthesis is that commercial laboratory apparatus favored significant rate enhancements compared to reactions, which run with conventional heating, (*i.e*. in oil bath)[15] and higher product yields.

The experiments were realized in the mono-mode microwave cavity of Monowave® 300 Anton-Paar apparatus operating with continuous power from 0 to 800 Watt at a frequency of 2.45 GHz and, the reactions were conducted in a glass reactor-vial closed with a snap-cap. Reaction optimization for the synthetic microwave process of compounds **6** consisted in varying the following parameters: - the nature of the solvent to obtain a homogeneous reaction mixture under microwave heating (toluene, DMF, DMSO, AcOEt, MeCN, pyridine, DIPEA, Et_3N), - the reaction time (20-45 min.), - and the reaction temperature (90-150 °C). After a complete screening of the reaction conditions, we observed a good reproducibility when we applied microwave irradiation during 30 min. at 120 °C from a stœchiometric mixture of reagents **4** and **5** in pyridine

(1 mmol./mL, total conc.). The desired 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6** was isolated from the crude reaction mixture after a simple precipitation from cooled AcOEt followed by successive washings with deionized water, diethyl ether and finally recrystallized from absolute ethanol to increase the quality of the precipitated product **6** before biological tests. Inspection of the data presented in Table 1 showed that this protocol for heterocyclocondensation under microwave irradiation can be applied to the 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** with commercial phenyl isothiocyanate **5** and the resulting 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** were synthesized in yields ranging (Table 1) from 55 to 75% and also in good overall yields (21-50%).

Table 1. Results for the preparation of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** and their precursors 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** and, 2-imino-2*H*-1-benzopyran-3-carbonitrile **3(a-f)** and, chemical shifts of CH_2 group for compounds **4(a-f)**, **6(a-f)** in DMSO-d_6 solution

Compound	R^1	R^2	R^3	R^4	Reaction time (min.)	Yield (%)[a]	Overall yield (%)[c]	δ for CH_2 (ppm)
3a	H	H	$N(Et)_2$	H	10	60	-	-
3b	H	H	H	H	120	80	-	-
3c	H	OMe	H	H	600	60	-	-
3d	H	H	OMe	H	60	80	-	-
3e	H	H	H	OMe	540	85	-	-
3f			H	H	20	96	-	-
4a	H	H	$N(Et)_2$	H	45	70	42	3.25
4b	H	H	H	H	45	82	66	3.45
4c	H	OMe	H	H	45	50	30	3.42
4d	H	H	OMe	H	45	90	63	3.37
4e	H	H	H	OMe	45	63	54	3.43
4f			H	H	45	95	91	3.77
6a	H	H	$N(Et)_2$	H	30	60[b]	25	3.56
6b	H	H	H	H	30	67[b]	44	3.73
6c	H	OMe	H	H	30	70[b]	21	3.72
6d	H	H	OMe	H	30	65[b]	41	3.64
6e	H	H	H	OMe	30	75[b]	41	3.72
6f			H	H	30	55[b]	50	3.94

[a] Isolated yield.

[b] Isolated yield after purification by recrystallization.

[c] Overall yield calculated from compound **3**.

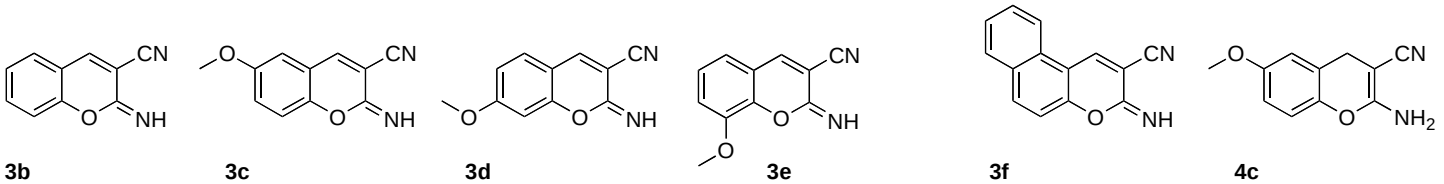
Before entering the biological tests, the six products **6** were characterized by ^1H, ^{13}C NMR, IR and HRMS. Examination of their ^1H NMR spectra in DMSO-d_6 solution showed signal for the CH_2 (H-5) in the range $3.56 < \delta < 3.96$ ppm. It is noteworthy that the NH of imino group (C-4 position) and thiourea function (N-1 position)

does not appear in the ^{1}H NMR spectra due to exchange with DMSO-d_6 solvent for analysis. The presence of the C-4 imino function of **6** in the IR spectrum was detected at 1623-1626 cm^{-1} and most important absorption bands were observed at 3133 and 3401 cm^{-1} (for **6a**) and attributed to the two N-H stretching frequencies. The presence of thiourea (C=S) and imino (C=NH) functions were confirmed respectively in the ^{13}C NMR spectra by a C-4 signal at δ 160.21 ppm and a C-2 signal at δ 179.14 ppm for **6a**. In mass spectrometry (MS) analysis, the $[M+Na]^+$ molecular ion signal for all products **6** were easily obtained as base signal.

Table 2. Effects of 2-imino-2*H*-1-benzopyran-3-carbonitrile **3(a-f)**, 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** and 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** on the catalytic activity of six purified protein kinases[a]

Compound	*Hs*CDK5-p25	GSK3α/β	CLK1	*Hs*Haspin	*Hs*Pim1	*Hs*Aurora B
3(a,b)	> 10	> 10	> 10	> 10	> 10	> 10
3c	> 10	> 10	**1.5**	> 10	> 10	> 10
3d	> 10	> 10	**0.25**	> 10	> 10	> 10
3e	> 10	> 10	**0.5**	> 10	> 10	> 10
3f, **4(a,b)**	> 10	> 10	> 10	> 10	> 10	> 10
4c	> 10	> 10	**0.9**	> 10	> 10	> 10
4(d-f), **6(a-f)**	> 10	> 10	> 10	> 10	> 10	> 10
Harmine [a]	8	19	0.071	0.59	> 10	NT [b]

Compounds were tested at various concentrations on each protein kinase as described in Supplementary Material. IC_{50} values, calculated from the dose-response curves, are reported in µM.
> 10, inhibitory but IC_{50} > 10 µM.
[a] Used as positive control.
[b] *NT* not tested.



3b Huh7: IC_{50} 2 µM; Caco2: IC_{50} 6 µM
3c Caco2: IC_{50} 6 µM; CLK1: IC_{50} 1.5 µM
3d CLK1: IC_{50} 0.25 µM
3e CLK1: IC_{50} 0.5 µM
3f Caco2: IC_{50} 9 µM; HCT 116: IC_{50} 9 µM
4c CLK1: IC_{50} 0.9 µM

Figure 2. Structure of 2-imino-2*H*-1-benzopyran-3-carbonitrile **3(b-e)**, 3-imino-3*H*-naphtho[2,1-*b*]pyran-2-carbonitrile **3f**, and 2-amino-6-methoxy-4*H*-1-chromene-3-carbonitrile (**4c**) which are active against tumor cell lines or protein kinase.

Exploring the biological properties of this new family of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** and their intermediates **3(a-f)**, **4(a-f)**, we tested these 18 products on six different *in vitro* kinase assays because these enzymes play an important role in protein phosphorylation of serine, threonine and tyrosine residues, all part of important cellular regulatory mechanism which are frequently deregulated in human diseases. The protein kinases used for these assays are respectively *Hs*CDK5-p25 (*Homo sapiens* cyclin dependant kinase 5p-25),[16] GSK3α/β (glycogene synthase kinase-3α/β),[17] CLK1 (cdc2-like kinase-1), *Hs*Haspin (*Homo sapiens* Haploid Germ Cell-Specific Nuclear Protein Kinase),[18] *Hs*Pim1

(*Homo sapiens* Pim1 proto-oncogene, serine/threonine kinase)[19] and *Hs*Aurora B. The IC_{50} values were determined from the dose-responses curves (Sigma Plot) and Harmine was used as reference for positive control. Results are given in Table 2. Analysis of the results for *in vitro* kinase assays highlighting three categories of compounds. The first category prove to be inactive products with IC_{50} > 10 µM and to our surprise, no 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6** exhibited inhibitory activity on protein kinases. The second category integrate compounds (Figure 2), which presents selective bioactivity in the micromolar range against CLK1. This is the case for iminocoumarin **3c** (IC_{50} 1.5 µM) and its 2-aminochromene derivative **4c** (IC_{50} 0.9 µM). The last category concerns compounds with selective submicromolar bioactivity against CLK1: this concerns **3e** (IC_{50} 0.5 µM) bearing a methoxy group in C-8 position and the most interesting compound is **3d** (with MeO in C-7 position) showed an IC_{50} 0.25 µM.

Table 3. Antiproliferative activity of 2-imino-2*H*-1-benzopyran-3-carbonitrile **3(a-f)**, 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** and 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** on seven representative tumor cell lines and fibroblasts

Compound	IC_{50} (µM) of selected compounds[a]							
	Huh7 D12	Caco 2	MDA-MB231	HCT 116	PC3	NCI-H727	HaCat	Fibroblasts
3a	> 25	18	48	19	32	42	20	> 25
3b	**2**	**6**	32	15	26	29	20	> 25
3c	> 25	**6**	> 25	18	39	43	24	> 25
3d	> 25	26	34	> 25	> 25	> 25	29	> 25
3e	> 25	27	> 25	24	> 25	> 25	32	> 25
3f	43	**9**	21	**9**	22	19	13	> 25
4(a-f)	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25
6(a-f)	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25
Roscovitine	15	15	12	9	13	43	11	> 25
Doxorubicine	0.03	0.04	0.01	0.03	34	65	0.02	> 25
Taxol	0.003	0.04	0.04	<0.001	<0.001	ND[b]	0.001	> 25
DMSO	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25

[a] IC_{50} values are expressed in µM and are the average of three assays, standard error ± 0.5 µM.
[b] ND: Not determined.

Continuing this biological exploration, these 18 compounds were also subjected to *in vitro* cancer assays[20, 21] against a panel of 7 tumor cell lines, HuH7-D12, Caco2, MDA-HB231, HCT116, PC3, NCI-H727, which are representative of different cancers (leukemia, melanoma and cancers of the liver, colon, breast, prostate, lung and kidney respectively). HaCat keratinocytes and diploid skin fibroblasts were also used as normal cell lines. Taxol, Doxorubicine and Roscovitine were also used as references for positive controls and their IC_{50} values are compared with those obtained for compounds **3(a-f)**, **4(a-f)** and **6(a-f)**. All results obtained were reported in Table 3. Again, none of compounds **6(a-f)** and also the 2-aminochromene **4(a-f)** presented a significant cytotoxic activity on the seven tumoral cell lines. For the other compounds **3(a-f)**, the most active compounds were clearly **3b**, **3c** and **3f** (Figure 2). They exhibited anti-tumor activities against the Huh7-D12, Caco2, HCT116 cell lines with IC_{50} values lower than 10 mM (Caco2, **3b**: IC_{50} 6 µM and **3f**: IC_{50} 9 µM; HCT116, **3f:** 9 µM, Huh7-D12, **3b**: IC_{50} 2 µM) and did not inhibit the growth of normal fibroblasts (IC_{50} > 25 µM).

Conclusions

This preliminary study described a new route to 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-d]pyrimidine-2(5*H*)-thione **6(a-f)** with good overall yields ranging from 21 to 50% using a solution-phase microwave dielectric heating protocol and 2-amino-4*H*-1-chromene-3-carbonitrile **4(a-f)** without substituent in C-4 position *via* iminocoumarins as starting materials. This protocol under microwave dielectric heating offered the possibility of an extension to a wide variety of substrates. The *in vitro* inhibition of cell proliferation was carried out on a panel of seven representative tumoral cell lines and the compounds **3(a-f)**, **4(a-f)** and **6(a-f)** were also evaluated against six protein kinases. Among all of these compounds, the iminocoumarin **3d** and the 2-amino-4*H*-1-chromene-3-carbonitrile **4c** turned out to be interesting because they presented selective submicromolar inhibition activity on CLK1 (**3d**: IC_{50} 0.25 µM and **4c**: IC_{50} 0.9 µM). The iminocoumarin **3b**, without substituent, exhibited antitumor activities against Huh7-D12 cell lines with IC_{50} 2 µM. These current results are the starting point of a new larger program within our group to investigate intensively the biological properties of these new inhibitors through a complete structure-activity relationship (SAR) with potential applications in cancer or diseases of the central nervous system, using the present protocol under microwave dielectric heating.

Experimental Section

General. Melting points were determined on a Kofler melting point apparatus and were uncorrected. Infrared (IR) spectra were registered on a Jasco FT-IR 420 spectrophotometer apparatus using KBr pellets. ^{1}H NMR spectra were recorded on BRUKER AC 300 P (300 MHz) spectrometer and ^{13}C NMR spectra on BRUKER AC 300 P (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 and on a Thermo Fisher Scientific Q-Exactive spectrometers at the "Centre Régional de Mesures Physiques de l'Ouest" SFS ScanMAT (CRMPO SFS ScanMAT, Rennes, France). Reactions under microwave irradiations were realized in the Anton Paar Monowave 300® microwave reactor (Anton Paar France) using borosilicate glass vials of 10 mL equipped with snap caps (at the end of the irradiation, cooling reaction was realized by compressed air). The microwave instrument consists of a continuous focused microwave power output from 0 to 800W for this Anton Paar Monowave 300® apparatus. All the experiments were performed using stirring option. The target temperature was reached with a ramp of 3 minutes and the chosen microwave power stay constant to hold the mixture at this temperature. The reaction temperature is monitored using calibrated infrared sensor and the reaction time included the ramp period. The microwave irradiation parameters (power and temperature) were monitored by the Monowave software package for the Anton Paar Monowave 300® reactor. All reagents and solvents were purchased from Acros Fisher or Sigma-Aldrich and were used without further purification. The 2-imino-2*H*-1-benzopyran-3-carbonitrile **3(a-c)** and **3f** were synthesized according to literature.[12]

Standard procedure for the synthesis of 2-imino-2*H*-1-benzopyran-3-carbonitriles 3(d,e). In a 50 mL two-necked round-bottomed flask, provided with magnetic stirrer and condenser, containing a solution of aromatic aldehyde 3 (10 mmol.) in absolute ethanol (20 mL) was added dropwise during 15 min. commercial malonitrile 2 (66.1 mg, 10 mmol.) in the presence of a catalytic amount of piperidine (0.5%). The resulting reaction mixture was stirred vigorously (500 rpm) for an appropriate reaction time at room temperature. Then, the reaction mixture was concentrated in a rotary evaporator under reduced pressure for elimination of

volatile solvent. The desired product 3 was collected by filtration on a Büchner funnel (porosity N°4) and washed with 3 x 10 mL of absolute ethanol. The desired 2-imino-2*H*-1-benzopyran-3-carbonitrile **3** was dried under high vacuum (10^{-2} Torr) at 25°C for 1 h that gave a yellowish powder and was further used without purification.

2-Imino-7-methoxy-2*H*-1-benzopyran-3-carbonitrile (3d). Compound **3d** was prepared in 80% yield (1.601 g) from 2-hydroxy-4-methoxy benzaldehyde **1e** (1.521 mg, 10 mmol.) according to the standard procedure and gave **3e** as yellowish powder, mp 162-164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.86 (s, 3H, OMe); 6.76 (s, 1H, H-8, Ar); 6.86 (d, 1H, *J* 9 Hz, H-6, Ar); 7.51 (d, 1H, *J* 9.1 Hz, H-5, Ar); 8.29 (s, 1H, H-4, Ar); 8.68 (br s, 1H, NH).

2-Imino-8-methoxy-2*H*-1-benzopyran-3-carbonitrile (3e). Compound **3e** was prepared in 85% yield (1.70 g) from 2-hydroxy-3-methoxy benzaldehyde **1e** (1.521 mg, 10 mmol.) according to the standard procedure and gave **3e** as yellowish powder, mp 173-175°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.87 (s, 3H, OCH_3); 7.20-7.24 (m, 3H, H-5, H-6, H-8, Ar); 8.34 (s, 1H, H-4, Ar); 8.92 (br s, 1H, NH).

General procedure for the synthesis of 2-amino-4*H*-1-chromene-3-carbonitrile 4(a-f) by reduction of 3-cyano 2-iminocoumarins 3(a-f). In a 50 mL round-bottomed flask, provided with a magnetic stirrer and condenser, containing a suspension of 2-imino-2*H*-1-benzopyran-3-carbonitrile **3** (12 mmol.) in 12 mL of methanol 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 min. After stirring, the mixture was poured in 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) . The desired 2-amino-4*H*[1]chromene-3-carbonitrile **4** was dried under high vacuum (10^{-2} Torr) at 25°C for 1 h that gave a yellowish powder and was further used without purification.

2-Amino-7-diethylamino-4*H*-1-chromene-3-carbonitrile (4a). Compound **4a** was prepared in 70% yield (2.92 g) from 7-diethylamino-2-imino-2*H*-1-benzopyran-3-carbonitrile **3a** (2.896 g, 12 mmol.) according to the standard procedure and gave **4a** as yellowish powder, mp 100-102 °C. IR (KBr, ν, cm^{-1}): 1653 (C=C), 2186 (CN), 3332-3467 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.04 (t, 6H, *J* 9.0 Hz, $C\underline{H}_3CH_2$); 3.25 (s, 2H, CH_2); 3.30 (q, 4H, *J* 9.0 Hz, $N(C\underline{H}_2CH_3)_2$); 6.12 (s, 1H, H-8, Ar); 6.42 (d, 1H, *J* 9.1 Hz, H-6, Ar); 6.61 (br s, 2H, NH_2); 6.92 (d, 1H, *J* 9.1 Hz, H-5, Ar). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 12.7 ($\underline{C}H_3CH_2$); 23.4 ($\underline{C}H_2$, C-4); 44.2 ($C\underline{H}_2N$); 49.9 (C-3, C**=**); 98.6 (C-8, Ar); 105.5 (C-6, Ar); 108.9 (C-4a, Ar); 121.8 (CN); 129.5 (C-5, Ar); 147.7 (C-7, Ar); 150.6 (C-8a, Ar); 161.3 (C-2, O-C=, Ar).

2-Amino-4*H*-1-chromene-3-carbonitrile (4b). Compound **4b** was prepared in 82% yield (1.69 g) from 2-imino-2*H*-1-benzopyran-3-carbonitrile **3b** (2.042 g, 12 mmol.) according to the standard procedure and gave **4b** as yellowish powder, mp 154-156 °C. IR (KBr, ν, cm^{-1}): 1664 (C=C), 2192 (CN), 3334-3447 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.45 (s, 2H, CH_2); 6.79 (br s, 2H, NH_2); 7.06-7.10 (m, 4H, H-5, H-6, H-7, H-8, Ar). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 24.1 ($\underline{C}H_2$, C-4); 49.4 (C-3, C=); 116.4 (C-8, Ar); 120.0 (CN); 121.5 (C-6, Ar); 124.9 (C-7, Ar); 128.4 (C-4a); 129.2 (C-5, Ar); 149.7 (C-8a, Ar); 161.3 (C-2, O-C=, Ar).

2-Amino-6-methoxy-4*H*-1-chromene-3-carbonitrile (4c). Compound **4c** was prepared in 55% yield (1.33 g) from 2-imino-6-methoxy-2*H*-1-benzopyran-3-carbonitrile **3c** (2.402 g, 12 mmol.) according to the standard procedure and gave **4a** as yellowish powder, mp 200-202 °C. IR (KBr, ν, cm^{-1}): 1658 (C=C), 2192 (CN), 3338-3405 (NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.42 (s, 2H, CH_2); 3.71 (s, 3H, MeO); 6.72 (br s, 2H, NH_2); 6.74 (s, 1H, H-5, Ar); 6.78 (d, 1H, *J* 9.0 Hz, H-7, Ar); 6.89 (d, 1H, *J* 9.0 Hz, H-8). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 24.5 ($\underline{C}H_2$, C-4); 48.9 ($\underline{C}H_3O$); 55.9 (C-3, C=); 113.2 (C-7, Ar); 114.1 (C-5, Ar); 117.2 (C-8, Ar); 120.9 (CN); 121.7 (C-4a, Ar); 143.6 (C-8a, Ar); 156.1 (C-6, Ar); 161.6 (C-2, O-C=, Ar).

2-Amino-7-methoxy-4*H*-1-chromene-3-carbonitrile (4d). Compound **4d** was prepared in 92% yield (2.22 g) from 2-imino-7-methoxy-2*H*-1-benzopyran-3-carbonitrile **3d** (2.402 g, 12 mmol.) according to the standard

procedure and gave **4d** as yellowish powder, mp 218-220 °C. IR (KBr, ν, cm^{-1}): 1666 (C=C), 2186 (CN), 3325-3425 (NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.37 (s, 2H, CH_2); 3.73 (s, 3H, MeO); 6.48 (s, 1H, H-8, Ar); 6.69 (d, 1H, *J* 9.0 Hz, H-6, Ar); 6.73 (br s, 2H, NH_2); 7.08 (d, 1H, *J* 9.1 Hz, H-5), Ar). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 23.5 ($\underline{C}H_2$, C-4); 49.8 ($\underline{C}H_3O$); 55.9 (C-3, C=); 101.6 (C-8, Ar); 111.2 (C-6, Ar); 111.6 (C-4a, Ar); 121.5 (CN); 129.8 (C-5, Ar); 150.2 (C-8a, Ar); 159.3 (C-7, Ar); 161.1 (C-2, O-C=, Ar).

2-Amino-8-methoxy-4*H*-1-chromene-3-carbonitrile (4e). Compound **4e** was prepared in 95% yield (2.30 g) from 2-imino-8-methoxy-2*H*-1-benzopyran-3-carbonitrile **3e** (2.402 g, 12 mmol.) according to the standard procedure and gave **4e** as yellowish powder, mp 152-154 °C. IR (KBr, ν, cm^{-1}): 1661 (C=C), 2186 (CN), 3332-3456 (NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ_H 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 (75 MHz, DMSO-d_6) δ_C 23.8 ($\underline{C}H_2$, C-4); 48.8 ($\underline{C}H_3O$); 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-4a, Ar); 138.5 (C-8a, Ar); 147.1 (C-8, Ar); 160.7 (C-2, O-C=, Ar).

2-Amino-4*H*-benzo[h]chromene-3-carbonitrile (4f). Compound **4f** was prepared in 90% yield (2.40 g) from 3-imino-3*H*-naphtho[2,1-*b*]pyran-2-carbonitrile **3f** (2.642 g, 12 mmol.) according to the standard procedure and gave **4f** as yellowish powder, mp 226-228 °C. IR (KBr, ν, cm^{-1}) 1675 (C=C), 2190 (CN), 3333-3443 (NH_2). ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.77 (s, 2H, CH_2); 6.88 (br s, 2H, NH_2); 7.17 (d, 1H, *J* 6.3 Hz, H-10, Ar); 7.54-7.58 (m, 2H, H-6, H-7, Ar); 7.75-7.79 (m, 2H, H-5, H-8, Ar); 7.93 (d, 1H, *J* 6.3 Hz, H-9, Ar). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 22.1 ($\underline{C}H_2$, C-4); 49.9 (C-3, C=); 112.3 (C-4a, Ar); 117.1 (C-10, Ar); 121.7 (CN); 123.4 (C-7, Ar); 125.5 (C-5, Ar); 127.7 (C-8a, Ar); 128.7 (C-6, Ar); 129.1 (C-8, C-9, Ar); 131.3 (C-4b, Ar); 146.7 (C-10a, Ar); 160.7 (C-2, O-C=, Ar).

General procedure under microwave irradiation for the synthesis of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione 6(a-f). In a 30 mL glass tube were placed successively 2-amino-4*H*[1]chromene-3-carbonitrile **4** (2.5 mmol.), commercial phenylisothiocyanate **5** (338 mg, 2.5 mmol.) and dry pyridine (5 mL) . The glass tube was sealed with a snap cap and placed in the Monowave® 300 Anton Paar microwave cavity (P = 800 Watt). The reaction mixture was irradiated at 120°C for 30 min. under vigorous magnetic stirring. After microwave dielectric heating, the crude reaction mixture was allowed to cool down at room temperature and 15 mL of cooled ethyl acetate was added in the glass tube, then mixing was pursued until complete precipitation. The resulting precipitate was collected by filtration on a Büchner funnel (porosity N°4) and washed successively with deionized water (5 x 10 mL) and diethyl ether (2 x 10 mL) . Then, this precipitate was dried firstly at 60°C at atmospheric pressure then, under high vacuum (10^{-2} Torr) at 25 °C for 1 hour. The desired product **6** was analyzed by ^1H, ^{13}C NMR and HRMS.

8-Diethylamino-4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione (6a). Compound **6a** was prepared in 60% yield (568 mg) from 2-amino-7-diethylamino-4*H*[1]chromene-3-carbonitrile **4a** (608.3 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6a** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1337 (C=S), 1619 (C=N), 3133 (NH), 3401 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ_H 1.09 (t, 6H, *J* 6.2 Hz, $C\underline{H}_3$); 3.33 (q, 4H, *J* 6.2 Hz, $N(C\underline{H}_2CH_3)_2$); 3.56 (s, 2H, $C\underline{H}_2$); 6.32 (s, 1H, H-9, Ar); 6.48 (d, 1H, *J* 9.0 Hz, H-7, Ar); 6.98 (d, 1H, *J* 9.0 Hz, H-6, Ar); 7.22 (d, 2H, *J* 9.0 Hz, H-2', Ar); 7.50-7.52 (m, 3H, H-3', H-4', Ar). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 12.8 ($N\underline{C}H_2$); 22.0 (C-5, $\underline{C}H_2$); 44.2 ($CH_2\underline{C}H_3$); 84.1 (C-4a, C=); 99.4 (C-9, Ar); 104.7 (C-5a, Ar); 108.1 (C-7, Ar); 128.3 (C-3', Ar); 129.5 (C-4', Ar); 129.9 (C-6, Ar); 130.6 (C-2', Ar); 139.2 (C-1', Ar); 147.9 (C-8, Ar); 151.1 (C-9b, O-C=, Ar); 156.9 (C-9a, Ar); 160.2 (C-4, C=N); 179.1 (C-2, C=S). ES^+ (MeOH/DCM 9:1) HRMS, *m/z*: 401.1415 found (calculated for $C_{21}H_{22}N_4OSNa$ $[M+Na]^+$ requires 401.1412).

4-Imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione (6b). Compound **6b** was prepared in 67% yield (515 mg) from 2-amino-4*H*[1]chromene-3-carbonitrile **4b** (430.5 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6b** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1344 (C=S), 1626 (C=N), 3109 (NH), 3395 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ_H 3.73 (s, 2H, H-5,

C$\underline{H}_2$); 7.15 (d, 2H, *J* 3.1 Hz, H-2', Ar); 7.20-7.24 (m, 4H, H-6, H-7, H-8, H-9, Ar); 7.57 (m, 3H, H-3', H-4', Ar). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C 22.8 (C-5, $\underline{C}H_2$); 83.5 (C-4a, Ar); 117.2 (C-5a, Ar); 119.3 (C-9, Ar); 125.0 (C-7, Ar); 127.0 (C-8, Ar); 129.1 (C-3', Ar); 129.6 (C-6, Ar); 129.6 (C-4', Ar); 130.7 (C-2', Ar); 139.2 (C-1', Ar); 150.3 (C-9b, O-C=, Ar); 156.9 (C-9a, Ar); 159.9 (C-4, C=N); 179.0 (C-2, C=S). ES$^+$ (MeOH/DCM 9:1) HRMS, *m/z*: 330.0680 found (calculated for $C_{17}H_{13}N_3OSNa$ [M+Na]$^+$ requires 330.0677).

4-Imino-7-methoxy-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione (6c). Compound **6c** was prepared in 70% yield (590 mg) from 2-amino-6-methoxy-4*H*[1]chromene-3-carbonitrile **4c** (505.5 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6c** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1346 (C=S), 1628 (C=N), 3073 (NH), 3370 (NH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ_H 3.72 (s, 2H, H-5, C$\underline{H}_2$); 3.76 (s, 3H, MeO); 6.71 (d, 1H, *J* 3.1 Hz, H-6, Ar); 6.86 (d, 1H, *J* 9.1 Hz, H-8, Ar); 7.08 (d, 1H, *J* 9.1 Hz, H-9, Ar); 7.22 (d, 2H, *J* 6.2 Hz, H-2'); 7.51-7.55 (m, 3H, H-3', H-4', Ar). ES$^+$ (MeOH/DCM 9:1) HRMS, *m/z*: 360.0782 found (calculated for $C_{18}H_{15}N_3O_2SNa$ [M+Na]$^+$ requires 360.0783).

4-Imino-8-methoxy-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione (6d). Compound **6d** was prepared in 65% yield (548 mg) from 2-amino-7-methoxy-4*H*[1]chromene-3-carbonitrile **4d** (505.5 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6d** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1347 (C=S), 1627 (C=N), 3084 (NH), 3373 (NH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ_H 3.64 (s, 2H, H-5, C$\underline{H}_2$); 3.78 (s, 3H, C$\underline{H}_3$O); 6.72 (d, 1H, H-9**,** Ar); 6.75 (d, 1H, *J* 9.0 Hz, H-7, Ar); 7.12 (d, 1H, *J* 9.0 Hz, H-6, Ar); 7.22 (d, 2H, *J* 9.0 Hz, H-2', Ar); 7.50-7.54 (m, 3H, H-3', H-4', Ar). ES$^+$ (MeOH/DCM 9:1) HRMS, *m/z*: 360.0782 found (calculated for $C_{18}H_{15}N_3O_2SNa$ [M+Na]$^+$ requires 360.0783).

4-Imino-9-methoxy-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione (6e). Compound **6e** was prepared in 75% yield (632 mg) from 2-amino-7-methoxy-4*H*[1]chromene-3-carbonitrile **4e** (505.5 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6e** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1344 (C=S), 1626 (C=N), 3120 (NH), 3424 (NH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ_H 3.72 (s, 2H, H-4, C$\underline{H}_2$); 3.85 (s, 3H, C$\underline{H}_3$O); 6.77 (d, 1H, *J* 6.1 Hz, H-8, Ar); 6.98 (d, 1H, *J* 9.1 Hz, H-6, Ar); 7.09 (dd, 1H, *J* 9.1 Hz, H-6, Ar); 7.24 (d, 2H, *J* 6.2 Hz, H-2', Ar); 7.51-7.55 (m, 3H, H-3', H-4', Ar). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C 22.9 (C-5, $\underline{C}H_2$); 56.3 ($\underline{C}H_3$O); 83.4 (C-4a, Ar); 111.5 (C-8, Ar); 120.0 (C-7, Ar); 120.7 (C-6, Ar); 128.9 (C-3', Ar); 129.6 (C-5a, Ar); 130.7 (C-2', Ar); 139.1 (C-1', Ar); 139.6 (C-9a, Ar); 148.2 (C-9, Ar); 157.0 (C-9b, O-C=, Ar); 159.6 (C-4, C=N); 179.0 (C-2, C=S). ES$^+$ (MeOH/DCM 9:1) HRMS, *m/z*: 360.0782 found (calculated for $C_{18}H_{15}N_3O_2SNa$ [M+Na]$^+$ requires 360.0783).

8,12-Dihydro-11-imino-10-phenyl-9*H*-naphtho[1',2':5,6]pyrano[2,3-*d*]pyrimidine-9-thione (6f). Compound **6f** was prepared in 55% yield (491 mg) from 2-amino-4*H*-benzo[h]chromene-3-carbonitrile **4f** (556 mg, 2.5 mmol.) according to the microwave irradiation general procedure and gave **6f** as yellowish powder. Mp > 260 °C. IR (KBr, ν, cm^{-1}): 1341 (C=S), 1623 (C=N), 3059 (NH), 3417 (NH). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ_H 3.94 (s, 2H, H-12, C$\underline{H}_2$); 7.60-7.64 (m, 11H, H-1, H-2, H-3, H-4, H-5, H-6, H-2', H-3', H-4', Ar). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ_C 21.4 (C-12, $\underline{C}H_2$); 83.6 (C-11a, Ar); 112.1 (C-12a, Ar); 117.9 (C-6, Ar); 123.9 (C-3, Ar); 125.5 (C-1, Ar); 127.6 (C-2, Ar); 128.8 (C-4, Ar); 128.9 (C-5, Ar); 129.0 (C-3', Ar); 129.2 (C-4', Ar); 129.6 (C-4a, Ar); 130.7 (C-2', Ar); 131.7 (C-12b, Ar); 139.2 (C-1', Ar); 147.4 (C-7a, O-C=, Ar); 157.2 (C-6a, Ar); 159.5 (C-4, C=N); 179.5 (C-2, C=S). ES$^+$ (MeOH/DCM 9:1) HRMS, *m/z*: 380.0831 found (calculated for $C_{21}H_{15}N_3OSNa$ [M+Na]$^+$ requires 380.0833).

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Supplementary Material

Cell culture and survival assays, kinase preparations and assays for biochemistry, ^{1}H NMR spectrum, ^{13}C NMR spectrum and IR of 4-imino-3-phenyl-3,4-dihydro-1*H*-chromeno[2,3-*d*]pyrimidine-2(5*H*)-thione derivatives **6(a-f)** are available on line at the supplementary materials.

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