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Anti-HIV and antiplasmodial activity of original flavonoid derivatives

Gilles Casano [a], Aurélien Dumètre [b], Christophe Pannecouque [c], Sébastien Hutter [b], Nadine Azas [b,*], Maxime Robin [a,*]

[a] Aix-Marseille Université, Institut des Sciences Moléculaires de Marseille, iSm2-UMR CNRS 6263, Centre Saint Jérôme, Service 552, 13397 Marseille Cedex 20, France
[b] UMR MD3 Relations Hôte-Parasites, Pharmacologie et Thérapeutique, Faculté de Pharmacie de Marseille 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France
[c] Rega Institute for Medical Research, Katholieke Universiteit Leuven, BP-3000 Leuven, Belgium

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ABSTRACT

In our search for potent anti-HIV and antiplasmodial agents, novel series of flavonoid derivatives and their chalcone intermediates were synthesized and evaluated for inhibition of HIV multiplication and antiproliferative activity on *Plasmodium falciparum* parasites. Chalcones exhibited a more selective antiplasmodial activity than flavonoids. Methoxyflavone **7e** was the only one compound active in both *P. falciparum* and HIV-1 whereas aminomethoxyflavones showed activity against HIV-2. Para substitution on the B ring seemed to increase HIV-2 potency.

1. Introduction

The HIV pandemic is still one of the most threatening infectious diseases despite many efforts to prevent transmission and improve anti-HIV chemotherapy. Currently available drugs do not completely eradicate replicating virus and resistant strains are emerging. HIV is especially widespread in sub-Saharan countries where it remains difficult to control because of many socio-economical reasons.

In the same countries, malaria, the most important parasitic diseases worldwide, leads to 212 million clinical cases and 800,000 deaths each year, mainly in children.[1] HIV/malaria co-infection is a risk factor of clinical malaria during pregnancy and severe malaria in children and adults.[2] Malaria management has become problematic because of the emergence of multidrug-resistant strains of *Plasmodium falciparum* and the lack of an effective vaccine.[3] Thus, one of the major challenges to roll back malaria is to find new active compounds structurally different to current antiplasmodial molecules for which resistance arises.[4]

Flavonoids are secondary metabolites widely distributed in plants. They fulfill many functions including flower pigmentation and protection against plant microorganisms and arthropods.[5] The flavonoid backbone derives from a chalcone intermediate and consists of two aromatic rings interconnected by a three carbon atom heterocyclic ring (Scheme 1). The subsequent modifications on this polyphenolic structure can lead to the great diversity of flavonoid derivatives including flavanones, flavones, isoflavones, flavonols, flavanols, catechins, and anthocyanines. Flavonoids are commonly found in human diet especially in fruits, vegetables, tea, red wine, and juices. Consumers and food manufacturers are interested in flavonoids because these compounds could exert direct or indirect beneficial effects on health.

Among their numerous biological activities including antioxidant, antitumor, and anti-inflammatory properties, some flavonoid derivatives were shown to be active on chloroquine-sensitive and resistant strains of *P. falciparum*.[6–9] Major flavonoids found in human diet, such as apigenin, luteolin and quercetin, can inhibit parasite growth by targeting metabolic pathways occurring in the apicoplast,[10–13] a plastid-like organelle encountered in apicomplexan parasites.[14] Besides a direct inhibition of the parasitic metabolic pathways, flavonoids could disturb the infected-erythrocyte cytoadherence properties and decrease their sequestration in small vessels.[15] Several flavonoids have an interesting anti-HIV activity,[7,16] for instance thalassiolin A and xanthohumol can inhibit several key enzymes involved in the HIV-1 replication.[17]

In order to evaluate the potential anti-HIV and antiplasmodial activity of flavonoid compounds, we have started a medicinal chemistry program to highlight a SAR study of chalcone and flavonoid derivatives with various substituents on the A, B, and C ring of

* Corresponding authors. Tel./fax: +33 491 835 564 (N.A.); tel.: +33 491 289 059; fax: +33 491 288 223 (M.R.).
E-mail addresses: nadine.azas@univmed.fr (N. Azas), maxime.robin@univ-cezanne.fr (M. Robin).

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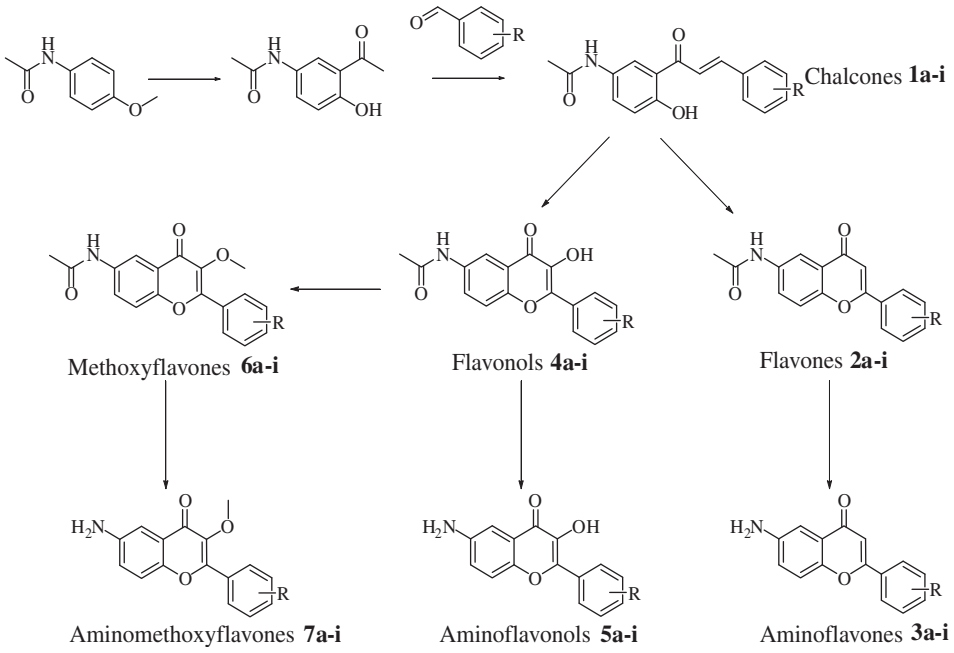

Scheme 1. Synthesis of chalcone and flavonoid derivatives.

flavonoids,[18] A ring bearing an acetamido or amino group in position 6, C ring with H, OH, and OMe in position 3 and mono substituents (H, OMe, F, CF_3) in various position of B ring (Scheme 1). To perform this SAR study, we have developed microwave irradiation (MWI) protocols.[19] A novel series of flavones and their corresponding chalcone intermediates were synthesized and evaluated against HIV-1 and HIV-2, and chloroquine-resistant *P. falciparum* parasites.

2. Results and discussion

2.1. Chemistry

Usually, aldol condensation between 2′-hydroxyacetophenone and aldehyde gives chalcone under strong basic condition in ethanol for at least 24 h in moderate yields.[20,21] So, we have adapted the Claisen–Schmidt condensation of substituted benzaldehydes[21] and 2-hydroxy-5-acetamidoacetophenone[22] to obtain our chalcone intermediates (**1a–i**) in good yields (70–95%) and short time (20 min) under microwave irradiation (MWI) (Table 1). Chalcones were converted to flavonols (**4a–i**) by treatment with alkaline hydrogen peroxide for 24 h, in yields of 40–70%.[23,24] These flavonols were methylated using dimethyl sulfate to obtain 4′ methoxy flavones (**6a–i**). Flavone derivatives (**2a–i**) were obtained by MWI of corresponding chalcone in DMSO with catalytic iodine in 90–95% yields. Deprotection of acetamido intermediates **2a–i**, **4a–i**, and **6a–i** to their corresponding amino derivatives (aminoflavones 3a–**i**, aminoflavonols **5a–i**, and methoxyaminoflavones **7a–i**) was done in ethanol/concentrated H_2SO_4 (9:1) under MWI for 30 min in quantitative yield.

2.2. Biological activity

The cytotoxicity, anti-HIV-1 IIIB, anti-HIV-2 ROD, and antiplasmodial activity of chalcone and flavonoid derivatives are reported in Table 2. Overall, chalcone intermediates (**1a–i**) have a more selective activity on *P. falciparum* than flavonoid derivatives (**2a–7i**). IC_{50}W2 of chalcones ranged from 6.7 to 16.9 μM and SI W2 from 1.3 to 10.2. Only two were cytotoxic for THP1 cells (IC_{50}THP1 ⩽ 15.5 μM). This fact was confirmed in the HIV assay with an homogeneous activity around 40 μM (IC_{50} on MT-4 cells ranging from 21.1 to 49.8 μM), but no selective activity was noted on both HIV-1 and HIV-2.

Among the flavonoid derivatives, compounds **2c**, **2i**, **3g**, **3i**, **4d**, **4h**, **5c**, **5g**, **5h**, and **6c** were highly toxic on THP1 cells (IC_{50}THP1 = 10 μM) (Table 2). A second class of derivatives included moderate cytotoxic but non specific compounds **2b**, **2g**, **3a**, **3e**, **3h**, **4a**, **4e–g**, **5a**, **5d–f**, **7d**, and **7h**. The most specific compounds (SIW2 3.1-8.3) were flavone **2e**, aminoflavone **3c**, methoxyflavones **6a**, **6b**, **6e**, **6g**, and **6i**, and aminomethoxyflavones **7a**, **7b**, and **7e** (Table 2). Methoxyflavone and aminomethoxyflavone derivatives exhibited the highest specific activity on *P. falciparum*

Table 1
Structure and yields of chalcone and flavonoid derivatives synthesized in this study

R	Chalcone		Flavone		Aminoflavone		Flavonol		Aminoflavonol		3-Methoxyflavone		Amino-3-methoxyflavone	
H	**1a**	97%	**2a**	92%	**3a**	84%	**4a**	41%	**5a**	94%	**6a**	86%	**7a**	80%
2′-OCH_3	**1b**	89%	**2b**	98%	**3b**	87%	**4b**	66%	**5b**	98%	**6b**	88%	**7b**	76%
3′-OCH_3	**1c**	85%	**2c**	89%	**3c**	91%	**4c**	54%	**5c**	98%	**6c**	84%	**7c**	91%
4′-OCH_3	**1d**	80%	**2d**	87%	**3d**	98%	**4d**	45%	**5d**	97%	**6d**	91%	**7d**	93%
2′-F	**1e**	98%	**2e**	87%	**3e**	98%	**4e**	52%	**5e**	98%	**6e**	82%	**7e**	82%
3′-F	**1f**	88%	**2f**	88%	**3f**	95%	**4f**	45%	**5f**	96%	**6f**	71%	**7f**	89%
4′-F	**1g**	84%	**2g**	90%	**3g**	94%	**4g**	40%	**5g**	96%	**6g**	86%	**7g**	91%
3′-CF_3	**1h**	95%	**2h**	98%	**3h**	96%	**4h**	26%	**5h**	94%	**6h**	85%	**7h**	77%
4′-CF_3	**1i**	91%	**2i**	81%	**3i**	95%	**4i**	30%	**5i**	95%	**6i**	93%	**7i**	86%

Table 2
Cytotoxicity, antiplasmodial, anti-HIV-1 and anti-HIV-2 activity of chalcone and flavonoid derivatives

Compound	IC_{50}[a] (μM)		SI W2[b]	CC_{50}[c] (μM)	IC_{50}[d] (μM)	SI[e]		
	THP1	W2		MT-4	HIV-1III_B	HIV-2 ROD	HIV-1III_B	HIV-2 ROD
1a	85.5	15.9	5.4	49.4	49.8	49.8	—	—
1b	>125	13.4	>9.3	37.8	37.8	37.8	—	—
1c	93.7	13.2	7.1	40	40	40	—	—
1d	59.2	6.7	8.8	38.7	38.7	38.7	—	—
1e	>125	12.3	>10.2	33.8	33.8	33.8	—	—
1f	78.6	15.7	5.0	30.1	30.1	30.1	—	—
1g	68.8	16.9	4.1	41.1	41.1	41.1	—	—
1h	8.4	11.3	—	32.1	32.1	32.1	—	—
1i	15.5	11.6	1.3	21.1	21.1	21.1	—	—
2a	97	>50	—	183.8	183.8	183.8	—	—
2b	50	>50	—	226	226	226	—	—
2c	10	>10	—	>404	>404	>404	—	—
2d	74	>50	—	225.8	225.8	225.8	—	—
2e	>125	40	3.1	205.3	205.3	205.3	—	—
2f	>125	50	>2.5	239.7	239.7	239.7	—	—
2g	22	>22	—	203.1	203.1	203.1	—	—
2h	50	50	—	10	10	10	—	—
2i	10	>10	—	329.9	329.9	329.9	—	—
3a	36	>36	—	209.1	209.1	209.1	—	—
3b	74	50	1.5	198.1	88	198.1	2.3	—
3c	>125	31	>4.0	247.2	247.2	247.2	—	—
3d	124	35	3.5	240.5	240.5	240.5	—	—
3e	44	>44	—	283.3	284.3	283.3	—	—
3f	>125	>50	—	268.7	61.1	58.4	4.4	4.6
3g	10	>10	—	264.2	264.2	264.2	—	—
3h	34	>34	—	137.6	137.6	137.6	—	—
3i	1.9	>1.9	—	273.2	273.2	273.2	—	—
4a	36	>36	—	64.7	64.7	64.7	—	—
4b	>125	50	2.5	384.6	384.6	384.6	—	—
4c	50	50	—	33.3	33.3	33.3	—	—
4d	9	>9	—	98.7	98.7	98.7	—	—
4e	47	>47	—	171.3	171.3	171.3	—	—
4f	36	>36	—	26.5	26.5	26.5	—	—
4g	26	>26	—	34.1	34.1	34.1	—	—
4h	1	>1	—	5.7	5.7	5.7	—	—
4i	>50	>50	—	7.3	7.3	7.3	—	—
5a	27	>27	—	216.5	216.5	216.5	—	—
5b	102	46	2.2	221.5	221.5	221.5	—	—
5c	2.0	>2.0	—	40.8	40.8	40.8	—	—
5d	29	>29	—	238.9	238.9	238.9	—	—
5e	38	>38	—	44.5	44.5	44.5	—	—
5f	30	>30	—	35.3	35.3	35.3	—	—
5g	4	>4	—	92.6	92.6	92.6	—	—
5h	2	>2	—	8.1	8.1	8.1	—	—
5i	>125	>50	—	3.9	3.9	3.9	—	—
6a	>125	18	>6.9	199.6	199.6	199.6	—	—
6b	108	16	6.7	153.6	153.6	153.6	—	—
6c	5	>5	—	368.7	368.7	368.7	—	—
6d	>50	21	>2.4	368.7	368.7	368.7	—	—
6e	>125	15	>8.3	180.3	180.3	180.3	—	—
6f	>125	49	2.6	209.6	209.6	209.6	—	—
6g	>125	23	>5.4	316.9	316.9	316.9	—	—
6h	52	19	2.7	200.7	200.7	200.7	—	—
6i	>125	39	>3.2	331.5	331.5	331.5	—	—
7a	74	20	3.7	126.1	126.1	126.1	—	—
7b	116	7.7	15.1	229	229	229	—	—
7c	37	13	2.8	250.6	250.6	250.6	—	—
7d	21	>21	—	200.2	200.2	58.2	—	3.4
7e	>125	23	>5.4	233.8	44.7	233.8	5.2	—
7f	34	>34	—	112.5	112.5	60.7	—	1.9
7g	36	33	1.1	140.6	140.6	140.6	—	—
7h	33	>33	—	73.1	50.4	57.6	1.5	1.3
7i	—	26	—	289.8	289.8	47.7	—	6.1
Doxorubicin	0.07							
Chloroquine	40	0.7	57					
Nevirapine				683	0.03	15	22,767	46
AZT				93.55	0.005		>17,990	
DDI				>529	5.37	2.71	>98	>195

[a] IC_{50}: compound concentration required to inhibit THP1 cell or W2 parasite proliferation by 50%.
[b] SI W2: selectivity index; ratio IC_{50}THP1/IC_{50}W2. —, SI ⩽ 1.
[c] CC_{50}: cytotoxic concentration of compound that reduces the normal uninfected MT-4 cell viability by 50%.
[d] IC_{50}: compound concentration required to inhibit HIV multiplication in MT-4 infected cells by 50%.
[e] SI: selectivity index; ratio CC_{50}/IC_{50}. —, SI ⩽ 1.

strain W2 (best compound **7b**, SI = 15.1), even if their SI was much lower than chloroquine (SI = 57). Investigating the subsequent substitutions on the phenyl ring B, fluor in position 2 promoted a specific antiplasmodial activity of methoxyflavone **6e** and aminomethoxyflavone **7e**.

In the case of HIV activity, there was no specificity for flavones (**2a–i**), aminoflavones (**3a–i**), flavonols (**4a–i**), aminoflavonols (**5a–i**), methoxyflavones (**6a–i**) (Table 2). Only six compounds (**3b**, **3f**, **7d**, **7e**, **7f**, **7i**) have slight activity against HIV-1 and/or 2 (Table 2). Aminoflavone **3f** was active on both HIV-1 and 2 (SI >4). **7e** was active against HIV 1 (SI >5) and was the only one that was active in both *P. falciparum* and HIV. Aminomethoxyflavones **7d**, **7f**, and **7i** exhibited inhibitory activities specifically against HIV-2 (ROD) with IC_{50} values in similar range (58.2 μM, 60.7 μM, and 47.7 μM, respectively). It was interesting to note that only amino derivatives show activity against HIV. Methoxyflavones were the most representatives, and para substitution on the B ring seems to increase HIV-2 potency. Such results on HIV-2 are promising because most of currently approved anti-HIV therapeutics has been designed against HIV-1 and not HIV-2.[25]

3. Conclusion

In this newly synthesized series of 54 flavonoid derivatives and their corresponding chalcones, we obtained a significant antiplasmodial activity of chalcones and methoxyflavone derivatives. Amino derivative **7e** was active both in *P. falciparum* and HIV-1 whereas compounds **7d**, **7f** and **7i** were specific inhibitors of the HIV-2 multiplication. Para substitution on the B ring is needed to promote antiplasmodial activity and increase HIV-2 potency.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined using a Bibby SMP3 apparatus and were uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer. All the experiments were carried out in DMSO-d_6 and the ^1H and ^{13}C chemical shifts of the solvent were used as a secondary reference and referred to the TMS signal from the usual relationships; the values of the chemical shifts (δ) are given in ppm and coupling constants (J) in Hertz. The numbering of the carbons is arbitrary. All chemicals used were of reagent grade, progress of the reaction was monitored by TLC on silica gel plates (Merck Silica Gel 60 F_{254}). Elemental analyses were performed by Spectropole (Campus St Jerome, Marseille, France) on a ThermoFinnigan FlashEA 1112 elemental analyzer, and the results were within ±0.4% of the theoretical values. Yields referred to purified products and were not optimized.

4.1.2. General procedure for the preparation of compounds (1a–i)

A solution of 2-hydroxy-5-acetamidoacetophenone (1.93 g, 10 mmol), benzaldehyde (10 mmol) and $LiOH{\cdot}H_2O$ (2.94 g, 70 mmol) in MeOH (20 mL) was submitted to microwave irradiation (MWI) in a CEM Apparatus. MWI Open vessel, power: 300 watts, solvent MeOH, $T°$ = 80 °C, 2 min and 20 min. The colorless solution was cooled to rt, MeOH was removed under vaccum and the mixture was poured into 1 N HCl (50 mL). The precipitate obtained was then filtered off, washed with excess water and dried to give chalcone (**1a**–**i**).

4.1.2.1. (*E*)-*N*-(3-Cinnamoyl-4-hydroxyphenyl) acetamide (1a). Yellow solid, yield: 97%, mol. wt: 292, mp: 190 °C.[20] Anal. Calcd for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.52; H, 5.38; N, 4.89. ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.03 (s, 3H, CH_3), 6.96 (d, 1H, J = 8.9 Hz, H_5), 7.47 (m, 3H, $H_{3'}$, $H_{4'}$, $H_{5'}$), 7.71 (dd, 1H, J = 2.5 Hz, 8.9 Hz, H_6), 7.77 (s, 2H, C-H_9, H_8), 7.79 (m, 2H, C-$H_{2'}$, $H_{6'}$), 8.17 (d, 1H, J = 2.5 Hz, H_2), 10.00 (s, NH), 11.70 (s, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.92 (CH_3), 117.90 (C-5), 121.17 (C-2), 121.46 (C-3), 122.94 (C-8), 128.22 (C-6), 128.99 (C-3′), 129.29 (C-2′), 131.13 (C-4′), 131.35 (C-1), 134.66 (C-1′), 144.30 (C-9), 156.93 (C-4), 168.35 (CO), 193.06 (C-7).

4.1.2.2. (*E*)-*N*-(4-Hydroxy-3-(3-(2-methoxyphenyl)acryloyl)phenyl)acetamide (1b). Yellow solid, yield: 89%, mol. wt: 311, mp: 168 °C. Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.32; H, 5.54; N, 4.47. ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 6.95 (d, 1H, J = 8.9 Hz, H_5), 7.05 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.13 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.48 (td, 1H, J = 1.5, 8.2 Hz, $H_{4'}$), 7.68 (dd, 1H, J = 2.5, 8.9 Hz, H_6), 7.78 (d, 1H, J = 8.2 Hz, $H_{6'}$), 7.85 (d, 1H, J = 15.7 Hz, H_8), 8.04 (d, 1H, J = 15.7 Hz, H_9), 8.26 (d, 1H, J = 2.5 Hz, H_2), 9.95 (s, 1H, NH), 11.91 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.85 (CH_3), 55.83 (OCH_3), 112.03 (C-3′), 117.83 (C-5), 120.56 (C-2), 120.90 (C-5′), 120.93 (C-3), 122.41 (C-8), 122.82 (C-1′), 127.97 (C-6′), 129.41 (C-6), 132.69 (C-4′), 131.27 (C-1), 139.51 (C-9), 157.17 (C-4), 158.69 (C-2′), 168.22 (CO), 193.22 (C-7).

4.1.2.3. (*E*)-*N*-(4-Hydroxy-3-(3-(3-methoxyphenyl)acryloyl)phenyl)acetamide (1c). Yellow solid, yield: 85%, mol. wt: 311, mp: 147 °C. Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.36; H, 5.49; N, 4.46. ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.02 (s, CH_3), 3.81 (s, OCH_3), 6.96 (d, 1H, J = 8.9 Hz, H_5), 7.04 (m, 1H, $H_{4'}$), 7.37 (m, 3H, $H_{2'}$, $H_{5'}$, $H_{6'}$), 7.71 (dd, 1H, J = 8.9, 2.5 Hz, H_6), 7.72 (d, 1H, J = 15.7 Hz, H_8), 7.79 (d, 1H, J = 15.7 Hz, H_9), 8.14 (d, 1H, J = 2.5 Hz, H_2), 9.92 (s, 1H, NH), 11.71 (s, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.88 (CH_3), 55.50 (OCH_3), 113.97 (C-2′), 116.93 (C-4′), 117.86 (C-5), 121.39 (C-3), 121.44 (C-6′), 123.26 (C-8), 128.29 (C-6), 130.29 (C-5′), 131.27 (C-1), 136.06 (C-1′), 144.28 (C-9), 156.93 (C-4), 159.87 (C-3′), 168.28 (CO), 193.13 (C-7).

4.1.2.4. (*E*)-*N*-(4-Hydroxy-3-(3-(4-methoxyphenyl) acryloyl) phenyl) acetamide (1d). Yellow solid, yield: 80%, mol. wt: 311, mp: 137 °C.[20] Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.41; H, 5.52; N, 4.46. ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.04 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 6.96 (d, 1H, J = 8.9 Hz, H_5), 7.04 (d, 2H, J = 8.7 Hz, $H_{3'}$), 7.64 (d, 1H, J = 15.7 Hz, H_8), 7.71 (dd, 1H, J = 2.5, 8.9 Hz, H_6), 7.78 (d, 2H, J = 8.7 Hz, $H_{2'}$), 7.79 (d, 1H, J = 15.7 Hz, H_9), 8.19 (d, 1H, J = 2.5 Hz, H_2), 9.95 (s, NH), 11.98 (s, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.72 (CH_3), 55.45 (OCH_3), 114.61 (C-3′), 117.69 (C-5), 119.57 (C-3), 120.89 (C-2, C-8), 127.03 (C-1′), 128.08 (C-6), 130.84 (C-2′), 131.03 (C-1), 144.55 (C-9), 157.10 (C-4), 161.71 (C-4′), 168.13 (CO), 192.89 (C-7).

4.1.2.5. (*E*)-*N*-(3-(3-(2-Fluorophenyl)acryloyl)-4-hydroxyphenyl) acetamide (1e). Yellow solid, yield: 98%, mol. wt: 299, mp: 179 °C. Anal. Calcd for $C_{17}H_{14}FNO_3$: C, 68.22; H, 4.71; N, 4.68. Found: C, 68.13; H, 4.70; N, 4.61. ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 6.96 (d, 1H, J = 8.9 Hz, H_5), 7.32 (m, 1H, $H_{5'}$), 7.34 (m, 1H, $H_{3'}$), 7.53 (m, 1H, $H_{4'}$), 7.71 (dd, 1H, J = 2.6, 8.9 Hz, H_6), 7.79 (d, 1H, J = 15.7 Hz, H_8), 7.85 (d, 1H, J = 15.7 Hz, H_9), 7.92 (td, 1H, J = 2.2, 7.6 Hz, $H_{6'}$), 8.14 (d, 1H, J = 2.6 Hz, H_2), 9.95 (s, 1H, NH), 11.57 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.74 (CH_3), 116.13–116.42 (d, J = 21.9 Hz, C-3′), 117.75 (C-5), 120.72 (C-2), 121.47 (C-3), 122.13–122.28 (d, J = 11.3 Hz, C-1′), 125.13–125.18 (d, J = 3.0 Hz, C-5′), 125.46 (C-8), 127.93 (C-6), 129.85–129.88 (d, J = 2.1 Hz, C-6′), 131.26 (C-1), 132.77–132.88

(d, J = 8.3 Hz, C-4′), 135.68 (C-9), 156.52 (C-4), 159.38–162.72 (d, J = 252 Hz, C-2′), 168.13 (CO), 192.45 (C-7).

4.1.2.6. (*E*)-*N*-(3-(3-(3-Fluorophenyl)acryloyl)-4-hydroxyphenyl) acetamide (1f). Yellow solid, yield: 88%, mol. wt: 299, mp: 169 °C. Anal. Calcd for $C_{17}H_{14}FNO_3$: C, 68.22; H, 4.71; N, 4.68. Found: C, 68.15; H, 4.73; N, 4.58. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.02 (s, 3H, CH_3), 6.96 (d, 1H, J = 8.9 Hz, H_5), 7.29 (td, 1H, J = 2.6, 8.6 Hz, $H_{4'}$), 7.50 (m, 1H, $H_{5'}$), 7.63 (d, 1H, J = 7.8 Hz, $H_{2'}$), 7.72 (m, 2H, $H_{6',6}$), 7.74 (d, 1H, J = 15.7 Hz, H_8), 7.82 (d, 1H, J = 15.7 Hz, H_9), 8.11 (d, 1H, J = 2.4 Hz, H_2), 9.87 (s, 1H, NH), 11.63 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.84 (CH_3), 114.83–115.12 (d, J = 21.9 Hz, C-2′), 117.52–117.80 (d, J = 21.1 Hz, C-4′), 117.87 (C-5), 121.46 (C-2), 121.46 (C-3), 121.46 (C-6′), 125.34 (C-8), 128.52 (C-6), 131.23 (C-1), 131.13–131.23 (d, J = 7.5 Hz, C-5′), 137.19–137.29 (d, J = 7.5 Hz, C-1′), 142.66 (C-9), 156.95 (C-4), 161.06–164.29 (d, J = 243 Hz, C-3′), 168.29 (CO), 193.00 (C-7).

4.1.2.7. (*E*)-*N*-(3-(3-(4-Fluorophenyl)acryloyl)-4-hydroxyphenyl) acetamide (1g). Yellow solid, yield: 84%, mol. wt: 299, mp: 153 °C. Anal. Calcd for $C_{17}H_{14}FNO_3$: C, 68.22; H, 4.71; N, 4.68. Found: C, 68.20; H, 4.68; N, 4.60. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.03 (s, 3H, CH_3), 6.97 (d, 1H, J = 8.9 Hz, H_5), 7.32 (t, 2H, J = 8.9 Hz, $H_{3'}$), 7.71 (dd, 1H, J = 2.5, 8.9 Hz, H_6), 7.73 (d, 1H, J = 15.7 Hz, H_8), 7.78 (d, 1H, J = 15.7 Hz, H_9), 7.90 (t, 2H, J = 8.9 Hz, $H_{2'}$), 8.18 (d, 1H, J = 2.5 Hz, H_2), 10.01 (s, 1H, NH), 11.73 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.70 (CH_3), 116.00–116.29 (d, J = 21.9 Hz, C-3′), 117.68 (C-5), 121.11 (C-2), 121.26 (C-3), 122.69 (C-8), 122.69 (C-1′), 128.10 (C-6), 131.12 (C-1), 131.12–131.25 (d, J = 9.8 Hz, C-2′), 142.87 (C-9), 156.74 (C-4), 161.92–165.22 (d, J = 249.0 Hz, C-4′), 168.13 (CO), 192.81 (C-7).

4.1.2.8. (*E*)-*N*-(4-Hydroxy-3-(3-(3-(trifluoromethyl)phenyl)acryloyl)phenyl)acetamide (1h). Yellow solid, yield: 95%, mol. wt: 349, mp: 169 °C. Anal. Calcd for $C_{18}H_{14}F_3NO_3$: C, 61.89; H, 4.04; N, 4.01. Found: C, 61.81; H, 4.09; N, 3.99. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.02 (s, 3H, CH_3), 6.97 (d, 1H, J = 8.9 Hz, H_5), 7.69 (t, 1H, J = 7.9 Hz, $H_{5'}$), 7.71 (dd, 1H, J = 2.6, 8.9 Hz, H_6), 7.82 (t, 1H, J = 7.9 Hz, $H_{4'}$), 7.83 (d, 1H, J = 15.7 Hz, H_8), 7.93 (d, 1H, J = 15.7 Hz, H_9), 8.10 (d, 1H, J = 2.6 Hz, H_2), 8.11 (br d, 1H, J = 7.9 Hz, $H_{6'}$), 8.21 (br s, 1H, $H_{2'}$), 9.91 (s, 1H, NH), 11.60 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.85 (CH_3), 117.87 (C-5), 121.48 (C-2), 121.68 (C-3), 125.28 (C-8), 124.20 (q, J = 270.00 Hz, CF_3), 125.36 (q, J = 4.2 Hz, C-2′), 127.08 (q, J = 4.2 Hz, C-4′), 128.54 (C-6), 130.08 (q, J = 31.7 Hz, C-3′), 130.29 (C-5′), 131.24 (C-1), 132.67 (C-6′), 135.89 (C-1′), 142.17 (C-9), 156.85 (C-4), 168.28 (CO), 192.93 (C-7).

4.1.2.9. (*E*)-*N*-(4-Hydroxy-3-(3-(4-(trifluoromethyl)phenyl)acryloyl)phenyl)acetamide (1i). Yellow solid, yield: 91%, mol. wt: 349, mp: 171 °C. Anal. Calcd for $C_{18}H_{14}F_3NO_3$: C, 61.89; H, 4.04; N, 4.01. Found: C, 61.78; H, 4.06; N, 4.02. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.02 (s, 3H, CH_3), 6.97 (d, 1H, J = 8.9 Hz, H_5), 7.70 (dd, 1H, J = 2.5, 8.9 Hz, H_6), 7.79 (d, 1H, J = 15.7 Hz, H_8), 7.82 (d, 2H, J = 8.2 Hz, $H_{2'}$), 7.89 (d, 1H, J = 15.7 Hz, H_9), 8.01 (d, 2H, J = 8.2 Hz, $H_{3'}$), 8.11 (d, 1H, J = 2.5 Hz, H_2), 9.92 (s, 1H, NH), 11.50 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.85 (CH_3), 117.87 (C-5), 121.28 (C-2), 121.78 (C-3), 124.09 (q, J = 269.0 Hz, CF_3), 126.04 (C-8), 126.05 (q, J = 3.8 Hz, C-3′), 128.27 (C-6), 129.43 (C-2′), 130.27 (q, J = 32.1 Hz, C-4′), 131.31 (C-1), 138.70 (C-1′), 141.79 (C-9), 156.60 (C-4), 168.24 (CO), 192.66 (C-7).

4.1.3. General procedure for the preparation of compounds (2a–i)

Chalcone **1a–i** (10 mmol), I_2 (0.28 g, 10% wt) were solubilized in DMSO (10 mL). The solution was submitted to MWI at 140 °C (DMSO, 2 + 20 min, open vessel). The solution was cooled to rt and poured onto cold 1 N HCl solution (100 mL). The solution was stirred for 1 h and the orange precipitate was then dilute with ice water (100 mL) and filtered off. The precipitate collected was washed with excess water to removed DMSO. The precipitate obtained was filtered off to give **2a–i** and used without further purification.

4.1.3.1. *N*-(4-Oxo-2-phenyl-4*H*-chromen-6-yl) acetamide (2a). Yellow solid, yield: 92%, mol. wt: 279, mp: 250.8 °C,[20] Anal. Calcd for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.02; H, 4.71; N, 5.00. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 7.00 (s, 1H, H_7), 7.58 (m, 1H, $H_{4'}$), 7.60 (m, 2H, $H_{3'}$), 7.75 (d, 1H, J = 9.0 Hz, H_4), 7.96 (dd, 1H, J = 2.7 Hz, 9.0 Hz, H_3), 8.10 (m, 2H, $H_{2'}$), 8.34 (d, 1H, J = 2.7 Hz, H_1), 10.27 (s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.98 (CH_3), 106.42 (C-7), 113.01 (C-1), 119.01 (C-4), 123.50 (C-8a), 125.56 (C-3), 126.29 (C-2′), 129.11 (C-3′), 131.21 (C-1′), 131.74 (C-4′), 136.79 (C-2), 151.49 (C-4a), 162.34 (C-6), 168.55 (CO), 176.98 (C-8).

4.1.3.2. *N*-(2-(2-Methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2b). Pale yellow solid, yield: 98%, mol. wt: 309. Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.79; H, 4.92; N, 4.46. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.06 (s, 3H, CH_3), 3.79 (s, 3H, $OCH_{3-2'}$), 6.84 (s, 1H, H_7), 7.10 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.20 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.52 (td, 1H, J = 8.2, 1.5 Hz, $H_{4'}$), 7.64 (d, 1H, J = 9.1 Hz, H_4), 7.85 (dd, 1H, J = 8.2, 1.5 Hz, $H_{6'}$), 7.89 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.30 (d, 1H, J = 2.5 Hz, H_1), 10.23 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 4.01 (CH_3), 55.98 ($OCH_{3-2'}$), 111.09 (C-3′), 112.59 (C-7), 112.94 (C-1), 118.96 (C-4), 119.99 (C-1′), 120.76 (C-5′), 123.25 (C-8a), 125.54 (C-3), 129.15 (C-6′), 132.86 (C-4′), 136.67 (C-2), 151.76 (C-4a), 157.58 (C-2′), 160.47 (C-6), 168.58 (CO), 177.02 (C-8).

4.1.3.3. *N*-(2-(3-Methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2c). Pale yellow solid, yield: 89%, mol. wt: 309, mp: 198 °C. Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.81; H, 4.88; N, 4.49. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.02 (s, 1H, H_7), 7.15 (m, 1H, $H_{4'}$), 7.47 (t, 1H, J = 7.9 Hz, $H_{5'}$), 7.57 (t, 1H, J = 2.4 Hz, $H_{2'}$), 7.64 (m, 1H, $H_{6'}$), 7.73 (d, 1H, J = 9.2 Hz, H_4), 7.94 (dd, 1H, J = 2.4, 9.0 Hz, H_3), 8.33 (d, 1H, J = 2.2 Hz, H_1), 10.30 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.02 (CH_3), 106.72 (C-7), 111.50 (C-2′), 113.01 (C-1), 117.59 (C-4′), 118.60 (C-6′), 119.10 (C-4), 123.51 (C-8a), 125.61 (C-3), 130.31 (C-5′), 132.64 (C-1′), 136.82 (C-2), 151.51 (C-4a), 159.79 (C-3′), 162.15 (C-6), 168.64 (CO), 177.08 (C-8).

4.1.3.4. *N*-(2-(4-Methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2d). Dark red solid, yield: 87%, mol. wt: 309, mp: 255 °C.[20] Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.84; H, 4.90; N, 4.52. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.90 (s, 1H, H_7), 7.12 (d, 2H, J = 8.9 Hz, $H_{3'}$), 7.72 (d, 1H, J = 9.1 Hz, H_4), 7.94 (d, 1H, J = 9.1, 2.5 Hz, H_3), 8.04 (d, 2H, J = 2.7 Hz, 9.0 Hz, $H_{2'}$), 8.31 (d, 1H, J = 2.5 Hz, H_1), 10.27 (s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.01 (CH_3), 55.56 (OCH_3), 105.00 (C-7), 113.13 (C-1), 114.61 (C-3′), 118.91 (C-4), 123.35 (C-1′, C-8a), 125.40 (C-3), 128.19 (C-2′), 136.69 (C-2), 151.46 (C-4a), 162.14 (C-6), 162.50 (C-4′), 168.61 (CO), 176.86 (C-8).

4.1.3.5. *N*-(2-(2-Fluorophenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2e). Black solid, yield: 87%, mol. wt: 297. Anal. Calcd for $C_{17}H_{12}FNO_3$: C, 68.68; H, 4.07; N, 4.71. Found: C, 68.61; H, 4.11; N, 4.69. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3), 6.70 (s, 1H, H_7), 7.40 (t, 1H, J = 7.8 Hz, $H_{5'}$), 7.42 (m, 1H, $H_{3'}$), 7.62

(m, 1H, $H_{4'}$), 7.65 (d, 1H, J = 9.1 Hz, H_4), 7.93 (dd, 1H, J = 2.6, 9.0 Hz, H_3), 7.95 (dd, 1H, J = 2.2, 7.6 Hz, $H_{6'}$), 8.31 (d, 1H, J = 2.6 Hz, H_1), 10.26 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.18 (CH_3), 111.01–111.14 (d, J = 8.3 Hz, C-6′), 113.11 (C-1), 116.94–117.23 (d, J = 22.6 Hz, C-3′), 119.24 (C-7), 119.81–119.89 (d, J = 14.3 Hz, C-1′), 123.47 (C-8a), 125.37 (C-5′), 125.97 (C-3), 129.75 (C-4), 133.71–133.84 (d, J = 6.8 Hz, C-1′), 137.12 (C-2), 151.84 (C-4a), 159.50 (C-6), 158.18–161.54 (d, J = 248.0 Hz, C-2′), 168.80 (CO), 176.93 (C-8).

4.1.3.6. *N*-(2-(3-Fluorophenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2f). Gray solid, yield: 88%, mol. wt: 297. Anal. Calcd for $C_{17}H_{12}FNO_3$: C, 68.68; H, 4.07; N, 4.71. Found: C, 68.64; H, 4.10; N, 4.67. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 7.06 (s, 1H, H_7), 7.43 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.61 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.73 (d, 1H, J = 9.0 Hz, H_4), 7.92 (br d, 1H, J = 8.0 Hz, $H_{6'}$), 7.93 (dd, 1H, J = 9.0, 2.7 Hz, H_3), 7.94 (dt, 1H, 11.1, 1.8 Hz, $H_{2'}$), 8.31 (d, 1H, J = 2.7 Hz, H_1), 10.27 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.00 (CH_3), 107.11 (C-7), 112.95 (C-1), 113.03–113.34 (d, J = 23.4 Hz, C-2′), 118.36–118.64 (d, J = 21.1 Hz, C-4′), 119.08 (C-4), 121.41–121.44 (d, J = 2.1 Hz, C-6′), 123.47 (C-8a), 125.64 (C-3), 131.16–131.27 (d, J = 8.3 Hz, C-5′), 133.56–133.66 (d, J = 7.5 Hz, C-1′), 136.89 (C-2), 151.41 (C-4a), 160.81 (C-6), 160.80–164.07 (d, J = 246 Hz, C-3′), 168.59 (CO), 177.02 (C-8).

4.1.3.7. *N*-(2-(4-Fluorophenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (2g). Brown solid, yield: 90%, mol. wt: 297, mp: 274 °C. Anal. Calcd for $C_{17}H_{12}FNO_3$: C, 68.68; H, 4.07; N, 4.71. Found: C, 68.66; H, 4.10; N, 4.65. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 6.97 (s, 1H, H_7), 7.40 (t, 2H, J = 8.9 Hz, $H_{3'}$), 7.71 (d, 1H, J = 9.1 Hz, H_4), 7.94 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.14 (dd, 2H, J = 8.9, 5.3 Hz, $H_{2'}$), 8.31 (d, 1H, J = 2.6 Hz, H_1), 10.26 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.98 (CH_3), 106.32 (C-7), 113.00 (C-1), 116.15–116.43 (d, J = 22.6 Hz, C-3′), 118.99 (C-4), 123.40 (C-8a), 125.55 (C-3), 127.80 (C-1′), 128.98 (d, J = 9.8 Hz, C-2′), 136.80 (C-2), 151.43 (C-4a), 161.40 (C-6), 162.34–165.25 (d, J = 250.00 Hz, C-4′), 168.56 (CO), 176.92 (C-8).

4.1.3.8. *N*-(4-Oxo-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (2h). Brown solid, yield: 98%, mol. wt: 347. Anal. Calcd for $C_{18}H_{12}F_3NO_3$: C, 62.25; H, 3.48; N, 4.03. Found: C, 62.21; H, 3.49; N, 3.98. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 7.18 (s, 1H, H_7), 7.80 (d, 1H, J = 8.9 Hz, H_4), 7.81 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.96 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.96 (br d, 1H, J = 8.2 Hz, $H_{4'}$), 8.32 (d, 1H, J = 2.6 Hz, H_1), 8.39 (br d, 1H, J = 8.2 Hz, $H_{6'}$), 8.40 (br s, 1H, $H_{2'}$), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.01 (CH_3), 107.50 (C-7), 112.96 (C-1), 119.21 (C-4), 122.90 (q, J = 3.8 Hz, C-2′), 123.42 (q, J = 270.00 Hz, CF_3), 123.51 (C-8a), 125.70 (C-3), 128.08 (q, J = 3.8 Hz, C-4′), 129.99 (q, J = 31.7 Hz, C-3′), 130.33 (C-5′, C-6′), 132.48 (C-1′), 136.94 (C-2), 151.49 (C-4a), 160.60 (C-6), 168.61 (CO), 177.04 (C-8).

4.1.3.9. *N*-(4-Oxo-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (2i). Brown solid, yield: 81%, mol. wt: 347, mp: 293 °C. Anal. Calcd for $C_{17}H_{12}FNO_3$: C, 62.25; H, 3.48; N, 4.03. Found: C, 62.18; H, 3.52; N, 3.95. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 7.15 (s, 1H, H_7), 7.78 (d, 1H, J = 9.1 Hz, H_4), 7.94 (d, 2H, J = 8.8 Hz, $H_{3'}$), 7.97 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.31 (d, 2H, J = 8.8 Hz, $H_{2'}$), 8.35 (d, 1H, J = 2.5 Hz, H_1), 10.30 (br s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 22.99 (CH_3), 107.84 (C-7), 112.94 (C-1), 119.12 (C-4), 123.50 (C-8a), 124.81 (q, J = 272 Hz, CF_3), 125.78 (C-3), 125.95 (q, J = 3.8 Hz, C-3′), 127.17 (C-2′), 131.27 (q, J = 32.4 Hz, C-4′), 135.19 (C-1′), 136.96 (C-2), 151.47 (C-4a), 160.62 (C-6), 168.61 (CO), 176.99 (C-8).

4.1.4. General procedure for deprotection of acetamido derivatives (3a–i)

Flavones **2a–i** (10 mmol) were added to a solution of EtOH (20 mL) and H_2SO_4 concd (5 mL). The solution was submitted to MWI in open vessel 80 °C, 2 + 20 min, upon cooling the solvent was removed under vacuo and the residue obtain is poured onto ice water (100 mL). Then the solution was neutralized with NH_4OH 16% until pH 7. The red precipitate formed was collected by filtration and washed with excess cold water. Amino compound was obtained as a red powder without further purification.

4.1.4.1. 6-Amino-2-phenyl-4*H*-chromen-4-one (3a). Brown solid, yield: 84%, mol. wt: 237, mp: 151 °C. Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.88; H, 4.72; N, 5.81. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 6.87 (s, 1H, H_7), 7.09 (dd, 1H, J = 8.8, 2.7 Hz, H_3), 7.13 (d, 1H, J = 2.7 Hz, H_1), 7.51 (d, 1H, J = 8.8 Hz, H_4), 7.57 (m, 1H, $H_{4'}$), 7.58 (m, 2H, $H_{3'}$), 8.05 (m, 2H, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 105.24 (C-1), 105.77 (C-7), 119.03 (C-4), 121.91 (C-3), 124.23 (C-8a), 126.11 (C-2′), 129.08 (C-3′), 131.43 (C-1′), 131.60 (C-4′), 146.23 (C-2), 148.08 (C-4a), 161.70 (C-6), 177.14 (C-8).

4.1.4.2. 6-Amino-2-(2-methoxyphenyl)-4*H*-chromen-4-one (3b). Brown solid, yield: 87%, mol. wt: 267. Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.84; H, 4.95; N, 5.21. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.90 (s, 3H, $OCH_{3-2'}$), 5.75 (br s, 2H, NH_2), 6.76 (s, 1H, H_7), 7.08 (dd, 1H, J = 8.9, 2.5 Hz, H_3), 7.12 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.14 (d, 1H, J = 2.5 Hz, H_1), 7.22 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.45 (d, 1H, J = 8.9 Hz, H_4), 7.54 (td, 1H, J = 8.2, 1.5 Hz, $H_{4'}$), 7.84 (dd, 1H, J = 8.2, 1.5 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.92 ($OCH_{3-2'}$), 105.30 (C-1), 110.52 (C-3′), 112.48 (C-7), 118.96 (C-4), 120.49 (C-1′), 120.76 (C-5′), 121.96 (C-3), 123.99 (C-8a), 129.06 (C-6′), 132.51 (C-4′), 145.95 (C-2), 148.45 (C-4a), 157.41 (C-2′), 159.91 (C-6), 177.13 (C-8).

4.1.4.3. 6-Amino-2-(3-methoxyphenyl)-4*H*-chromen-4-one (3c). Brown solid, yield: 91%, mol. wt: 267. Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.81; H, 4.91; N, 5.25. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.86 (s, 3H, OCH_3), 6.91 (s, 1H, H_7), 7.09 (dd, 1H, J = 2.8, 8.9 Hz, H_3), 7.12 (d, 1H, J = 8.9 Hz, H_1), 7.15 (m, 1H, $H_{4'}$), 7.47 (t, 1H, J = 7.9 Hz, $H_{5'}$), 7.53 (d, 1H, J = 9.1 Hz, H_4), 7.55 (t, 1H, J = 2.3 Hz, $H_{2'}$), 7.62 (m, 1H, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.75 (OCH_3), 105.31 (C-1), 106.08 (C-7), 111.31 (C-2′), 117.24 (C-4′), 118.40 (C-6′), 119.11 (C-4), 122.07 (C-3), 124.23 (C-8a), 130.22 (C-5′), 133.01 (C-1′), 145.85 (C-2), 148.21 (C-4a), 159.69 (C-3′), 161.48 (C-6), 177.16 (C-8).

4.1.4.4. 6-Amino-2-(4-methoxyphenyl)-4*H*-chromen-4-one (3d). Dark brown solid, yield: 80%, mol. wt: 267, mp: 147 °C.[20] Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.83; H, 4.97; N, 5.19. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.85 (s, OCH_3), 5.49 (s, NH_2), 6.78 (s, 1H, H_7), 7.06 (dd, 1H, J = 8.8, 2.7 Hz, H_3), 7.08 (d, 1H, J = 2.7 Hz, H_1), 7.10 (d, 2H, J = 8.9 Hz, $H_{3'}$), 7.47 (d, 1H, J = 8.8 Hz, H_4), 8.00 (d, 2H, J = 8.9 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.49 (OCH_3), 104.38 (C-1), 105.01 (C-7), 114.51 (C-3′), 118.83 (C-4), 121.45 (C-3), 123.78 (C-1′), 124.18 (C-8a), 127.89 (C-2′), 146.55 (C-2), 147.81 (C-4a), 161.82 (C-6, C-4′), 177.03 (C-8).

4.1.4.5. 6-Amino-2-(2-fluorophenyl)-4*H*-chromen-4-one (3e). Dark brown solid, yield: 98%, mol. wt: 255. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.43; H, 3.98; N, 5.51. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 6.93 (s, 1H, H_7), 5.55 (s, 2H, NH_2), 7.09

(dd, 1H, J = 2.7, 8.8 Hz, H_3), 7.11 (d, 1H, J = 2.7 Hz, H_1), 7.41 (m, 1H, $H_{5'}$), 7.44 (m, 1H, $H_{3'}$), 7.46 (d, 1H, J = 8.8 Hz, H_4), 7.65 (m, 1H, $H_{4'}$), 7.95 (td, 1H, J = 7.6, 2.1 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 104.72 (C-1), 110.18–110.30 (d, J = 9.0 Hz, C-7), 116.73–116.02 (d, J = 21.9 Hz, C-3′), 119.01 (C-4), 120.03–120.16 (d, J = 9.8 Hz, C-1′), 121.94 (C-3), 124.05 (C-8a), 125.19 (C-5′), 129.47 (C-6′), 132.23–132.35 (d, J = 9.1 Hz, C-4′), 146.85 (C-2), 148.09 (C-4a), 157.72 (C-6), 157.90–161.26 (d, J = 253.00 Hz, C-2′), 176.84 (C-8).

4.1.4.6. 6-Amino-2-(3-fluorophenyl)-4*H*-chromen-4-one (3f). Brown solid, yield: 95%, mol. wt: 255, mp: 122 °C. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.43; H, 3.98; N, 5.51. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 6.95 (s, 1H, H_7), 7.07 (dd, 1H, J = 9.0, 2.7 Hz, H_3), 7.10 (br s, 2H, NH_2), 7.11 (d, 1H, J = 2.7 Hz, H_1), 7.42 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.52 (d, 1H, J = 9.0 Hz, H_4), 7.61 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.91 (br d, 1H, J = 8.0 Hz, $H_{6'}$), 7.91 (dt, 1H, J = 11.1, 1.8 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 104.83 (C-1), 106.43 (C-7), 112.83–113.14 (d, J = 23.3 Hz, C-2′), 118.03–118.16 (d, J = 20.4 Hz, C-4′), 119.08 (C-4), 121.88 (C-3), 122.26 (C-6′), 124.23 (C-8a), 131.14–131.25 (d, J = 8.3 Hz, C-5′), 133.99–134.11 (d, J = 8.9 Hz, C-1′), 146.72 (C-2), 147.47 (C-4a), 160.13 (C-6), 164.08–167.16 (d, J = 246 Hz, C-3′), 177.18 (C-8).

4.1.4.7. 6-Amino-2-(4-fluorophenyl)-4*H*-chromen-4-one (3g). Brown solid, yield: 94%, mol. wt: 255. Anal. Calcd for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 70.38; H, 4.03; N, 5.42. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.08 (s, 1H, H_7), 7.45 (t, 2H, J = 8.8 Hz, $H_{3'}$), 7.63 (dd, 1H, J = 9.0, 2.6 Hz, H_3), 7.82 (d, 1H, J = 2.6 Hz, H_1), 7.87 (d, 1H, J = 9.0 Hz, H_4), 8.20 (dd, 2H, J = 8.8, 5.4 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 106.56 (C-7), 115.81 (C-1), 116.16–116.46 (d, J = 22.6 Hz, C-3′), 120.32 (C-4), 123.85 (C-4a), 127.47 (C-3), 127.60 (C-1′), 129.10–129.22 (d, J = 9.0 Hz, C-2′), 133.34 (C-2), 153.09 (C-4a), 161.86 (C-6), 162.59–165.91 (d, J = 250.00 Hz, C-4′), 176.55 (C-8).

4.1.4.8. 6-Amino-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (3h). Brown solid, yield: 96%, mol. wt: 305. Anal. Calcd for $C_{16}H_{10}F_3NO_2$: C, 62.96; H, 3.30; N, 4.59. Found: C, 62.89; H, 3.35; N, 4.42. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.06 (s, 1H, H_7), 7.11 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.13 (d, 1H, J = 2.6 Hz, H_1), 7.57 (d, 1H, J = 8.9 Hz, H_4), 7.80 (t, 1H, J = 7.9 Hz, $H_{5'}$), 7.94 (t, 1H, J = 7.9 Hz, $H_{4'}$), 8.35 (br s, 1H, $H_{2'}$), 8.36 (br d, 1H, J = 7.9 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 105.31 (C-1), 106.98 (C-7), 119.38 (C-4), 122.82 (q, J = 3.8 Hz, C-2′), 122.26 (C-3), 124.43 (C-8a), 124.05 (q, J = 270.00 Hz, CF_3), 127.92 (q, J = 3.8 Hz, C-4′), 130.11 (q, J = 32.4 Hz, C-3′), 130.31 (C-5′), 130.48 (C-6′), 133.02 (C-1′), 146.57 (C-2), 160.07 (C-6), 148.25 (C-4a), 177.34 (C-8).

4.1.4.9. 6-Amino-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (3i). Brown solid, yield: 95%, mol. wt: 305. Anal. Calcd for $C_{16}H_{10}F_3NO_2$: C, 62.96; H, 3.30; N, 4.59. Found: C, 62.88; H, 3.31; N, 4.52. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.08 (s, 1H, H_7), 7.29 (dd, 1H, J = 2.5, 9.1 Hz, H_3), 7.36 (d, 1H, J = 2.5 Hz, H_1), 7.65 (d, 2H, J = 9.1 Hz, H_4), 7.93 (d, 2H, J = 8.8 Hz, $H_{3'}$), 8.29 (d, 2H, J = 8.8 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 107.46 (C-7), 108.75 (C-1), 119.59 (C-4), 124.06 (C-3), 124.17 (C-8a), 124.81 (q, J = 272 Hz, CF_3), 125.98 (q, J = 3.8 Hz, C-3′), 130.95 (C-2′), 129.25 (q, J = 32.4 Hz, C-4′), 135.40 (C-1′), 142.06 (C-2), 149.80 (C-4a), 160.07 (C-6), 176.98 (C-8).

4.1.5. General procedure for synthesis of flavonols (4a–i)

Chalcones **1a–i** (10 mmol) was solubilized in EtOH (50 mL), then was added NaOH 5% (40 mL) and H_2O_2 25% (10 mL). The solution was stirred overnight at rt and put onto water (100 mL) and acidified with 2 M HCl. The yellow precipitate obtained was filtered, washed with water and dried to give **4a–i**.

4.1.5.1. *N*-(3-Hydroxy-4-oxo-2-phenyl-4*H*-chromen-6-yl)acetamide (4a)[26]. Yellow solid, yield: 41%, mol. wt: 295. Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.12; H, 4.48; N, 4.68. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 7.51 (m, 1H, $H_{4'}$), 7.55 (m, 2H, $H_{3'}$), 7.71 (d, 1H, J = 9.2 Hz, H_4), 7.90 (dd, 1H, J = 2.5 Hz, 9.2 Hz, H_3), 8.19 (m, 2H, $H_{2'}$), 8.42 (d, 1H, J = 2.5 Hz, H_1), 9.53 (s, OH), 10.30 (s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.09 (CH_3), 112.77 (C-1), 119.08 (C-4), 121.48 (C-8a), 125.74 (C-3), 127.75 (C-2′), 128.68 (C-3′), 130.02 (C-1′), 131.43 (C-4′), 136.05 (C-2), 138.87 (C-7), 145.25 (C-6), 150.76 (C-4a), 168.84 (CO), 172.96 (C-8).

4.1.5.2. *N*-(3-Hydroxy-2-(2-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (4b). White solid, yield: 66%, mol. wt: 325. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.42; H, 4.71; N, 4.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 2H, CH_3), 3.79 (s, 3H, $OCH_{3-2'}$), 7.08 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.19 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.48 (t, 1H, J = 8.2 Hz, $H_{4'}$), 7.49 (d, 1H, J = 8.2 Hz, $H_{6'}$), 7.58 (d, 1H, J = 9.1 Hz, H_4), 7.86 (dd, 1H, J = 9.1, 2.6 Hz, H_3), 8.46 (d, 1H, J = 2.6 Hz, H_1), 8.87 (s, 1H, OH), 10.27 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.02 (CH_3), 55.76 (OCH_3), 111.96 (C-3′), 112.71 (C-1), 118.89 (C-4), 119.94 (C-1′), 120.15 (C-5′), 122.06 (C-8a), 125.26 (C-3), 131.06 (C-6′), 131.75 (C-4′), 135.89 (C-2), 138.81 (C-7), 147.00 (C-6), 151.05 (C-4a), 157.13 (C-2′), 168.56 (CO), 172.56 (C-8).

4.1.5.3. *N*-(3-Hydroxy-2-(3-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (4c). Orange solid, yield: 54%, mol. wt: 325. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.42; H, 4.61; N, 4.30. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 7.09 (br d, 1H, J = 8.6 Hz, $H_{4'}$), 7.47 (t, 1H, J = 8.6 Hz, $H_{5'}$), 7.73 (d, 1H, J = 9.0 Hz, H_4), 7.75 (t, 1H, J = 1.8 Hz, $H_{2'}$), 7.77 (br d, 1H, J = 8.6 Hz, $H_{6'}$), 7.92 (dd, 1H, J = 9.0, 2.5 Hz, H_3), 8.45 (d, 1H, J = 2.5 Hz, H_1). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.05 (CH_3), 55.36 (OCH_3), 112.71 (C-1), 113.46 (C-2′), 115.31 (C-4′), 119.05 (C-4), 120.10 (C-6′), 121.37 (C-8a), 125.76 (C-3), 129.77 (C-5′), 132.65 (C-1′), 136.09 (C-2), 138.97 (C-7), 144.86 (C-6), 150.68 (C-4a), 159.25 (C-3′), 168.82 (CO), 172.94 (C-8).

4.1.5.4. *N*-(3-Hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (4d). Orange solid, yield: 45%, mol. wt: 325. Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.41; H, 4.64; N, 4.22. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 7.12 (m, 2H, $H_{3'}$), 7.71 (d, 1H, J = 9.1 Hz, H_4), 7.88 (dd, 1H, J = 2.5, 9.1 Hz, H_3), 8.19 (d, 2H, J = 9.1 Hz, $H_{2'}$), 8.41 (d, 1H, H_1), 9.40 (s, OH), 10.26 (s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.17 (CH_3), 55.53 (OCH_3), 112.81 (C-1), 113.22 (C-3′), 118.98 (C-4), 123.26 (C-8a), 124.43 (C-1′), 125.43 (C-3), 129.53 (C-2′), 136.06 (C-2), 138.05 (C-7), 145.65 (C-6), 150.62 (C-4a), 160.58 (C-4′), 168.70 (CO), 172.62 (C-8).

4.1.5.5. *N*-(2-(2-Fluorophenyl)-3-hydroxy-4-oxo-4*H*-chromen-6-yl)acetamide (4e). White solid, yield: 52%, mol. wt: 313, mp: 210 °C. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.11; H, 3.89; N, 4.43. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 3H, CH_3), 7.37 (m, 1H, $H_{5'}$), 7.42 (m, 1H, $H_{3'}$), 7.59 (m, 1H, $H_{4'}$), 7.62 (d, 1H, J = 8.8 Hz, H_4), 7.75 (td, 1H, J = 7.6, 2.2 Hz, $H_{6'}$), 7.88 (dd, 1H, J = 2.7, 8.8 Hz, H_3), 8.48 (d, 1H, J = 8.8 Hz, H_1), 9.36 (s, 1H, OH), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.03 (CH_3), 112.72 (C-1), 116.03–116.31 (d, J = 21.1 Hz, C-3′), 118.95 (C-4), 118.95–119.16 (J = 14.3 Hz, C-1′), 121.96 (C-8a), 124.40 (C-5′), 125.57 (C-3), 131.22 (C-6′), 132.42–132.54 (d, J = 9.0 Hz, C-4′), 136.08 (C-2), 139.22 (C-7), 143.36 (C-6), 151.05 (C-4a), 157.52–160.85 (d, J = 251.00 Hz, C-2′), 168.61 (CO), 172.61 (C-8).

4.1.5.6. *N*-(2-(3-Fluorophenyl)-3-hydroxy-4-oxo-4*H*-chromen-6-yl)acetamide (4f). Yellow solid, yield: 45%, mol. wt: 313. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.17; H, 3.79; N, 4.42. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 3H, CH_3), 7.10 (br s, 1H, NH), 7.33 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.60 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.73 (d, 1H, J = 9.1 Hz, H_4), 7.92 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.02 (dt, 1H, J = 11.1, 1.8 Hz, $H_{2'}$), 8.07 (br d, 1H, J = 8.0 Hz, $H_{6'}$), 8.45 (d, 1H, J = 2.5 Hz, H_1). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.99 (CH_3), 112.59 (C-1), 114.24–114.30 (d, J = 24.1 Hz, C-2′), 116.65–116.71 (d, J = 20.4 Hz, C-4′), 118.99 (C-4), 121.31 (C-6′), 123.59 (C-8a), 125.81 (C-3), 130.59–130.70 (d, J = 8.3 Hz, C-5′), 133.55–133.67 (d, J = 8.9 Hz, C-1′), 136.10 (C-2), 139.32 (C-7), 143.39 (C-6), 150.58 (C-4a), 160.43–163.64 (d, J = 242 Hz, C-3′), 168.67 (CO), 177.97 (C-8).

4.1.5.7. *N*-(2-(4-Fluorophenyl)-3-hydroxy-4-oxo-4*H*-chromen-6-yl)acetamide (4g). Yellow solid, yield: 40%, mol. wt: 313. Anal. Calcd for $C_{17}H_{12}FNO_4$: C, 65.18; H, 3.86; N, 4.47. Found: C, 65.12; H, 3.89; N, 4.41. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 7.40 (t, 2H, J = 9.0 Hz, $H_{3'}$), 7.71 (d, 1H, J = 9.0 Hz, H_4), 7.91 (dd, 1H, J = 9.0, 2.5 Hz, H_3), 8.27 (dd, 2H, J = 9.0, 5.4 Hz, $H_{2'}$), 8.44 (d, 1H, J = 2.5 Hz, H_1), 10.35 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.00 (CH_3), 112.63 (C-1), 115.48–115.76 (d, J = 21.0 Hz, C-3′), 118.90 (C-4), 121.37 (C-8a), 125.55 (C-3), 128.00 (C-1′), 130.01–130.12 (d, J = 8.0 Hz, C-2′), 136.01 (C-2), 138.86 (C-7), 144.28 (C-6), 150.55 (C-4a), 160.95–164.24 (d, J = 247.00 Hz, C-4′), 168.62 (CO), 173.00 (C-8).

4.1.5.8. *N*-(3-Hydroxy-4-oxo-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (4h). Yellow solid, yield: 26%, mol. wt: 363. Anal. Calcd for $C_{18}H_{12}F_3NO_4$: C, 59.51; H, 3.33; N, 3.86. Found: C, 59.45; H, 3.35; N, 3.83. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 7.76 (d, 1H, J = 8.9 Hz, H_4), 7.80 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.85 (br d, 1H, J = 8.2 Hz, $H_{4'}$), 7.91 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 8.42 (d, 1H, J = 2.6 Hz, H_1), 8.46 (br d, 1H, J = 8.2 Hz, $H_{6'}$), 8.54 (br s, 1H, $H_{2'}$), 9.93 (br s, 1H, OH), 10.25 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.98 (CH_3), 112.57 (C-1), 119.04 (C-4), 121.36 (C-8a), 123.42 (q, J = 270.00 Hz, CF_3), 123.96 (q, J = 3.8 Hz, C-2′), 125.73 (C-3), 126.03 (q, J = 3.8 Hz, C-4′), 129.33 (q, J = 31.7 Hz, C-3′), 129.78 (C-5′), 131.10 (C-6′), 132.45 (C-1′), 136.07 (C-2), 139.44 (C-7), 143.11 (C-6), 150.59 (C-4a), 168.55 (CO), 172.95 (C-8).

4.1.5.9. *N*-(3-Hydroxy-4-oxo-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (4i). Yellow solid, yield: 30%, mol. wt: 363. Anal. Calcd for $C_{18}H_{12}F_3NO_4$: C, 59.51; H, 3.33; N, 3.86. Found: C, 59.43; H, 3.38; N, 3.82. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 7.73 (d, 1H, J = 8.9 Hz, H_4), 7.90 (dd, 1H, J = 2.5, 8.9 Hz, H_3), 7.92 (d, 2H, J = 8.9 Hz, $H_{3'}$), 8.40 (d, 2H, J = 8.9 Hz, $H_{2'}$), 8.44 (d, 1H, J = 2.5 Hz, H_1), 9.98 (s, 1H, OH), 10.26 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.19 (CH_3), 112.76 (C-1), 119.21 (C-4), 121.53 (C-8a), 124.68 (q, J = 272.0 Hz, CF_3), 125.59 (q, J = 3.8 Hz, C-3′), 126.02 (C-3), 128.31 (C-2′), 130.50 (q, J = 32.4 Hz, C-4′), 135.58 (C-1′)136.32 (C-2), 150.82 (C-4a), 143.41 (C-6), 168.76 (CO), 172.65 (C-8).

4.1.6. General procedure for deprotection of acetamido derivatives (5a–i)

Flavonol **4a–i** (10 mmol) was added to a solution of EtOH (20 mL) and H_2SO_4 concd (5 mL). The solution was submitted to MWI in open vessel 80 °C, 2 + 20 min, upon cooling the solvent was removed under vacuo and the residue obtain is poured onto ice water (100 mL). Then the solution was neutralized with NH_4OH 16% until pH 7. The red precipitate formed was collected by filtration and washed with excess cold water. Amino compound was obtained as a red powder without further purification.

4.1.6.1. 6-Amino-3-hydroxy-2-phenyl-4*H*-chromen-4-one (5a). White solid, yield: 94%, mol. wt: 253. Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.11; H, 4.42; N, 5.49. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.10 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.18 (d, 1H, J = 2.6 Hz, H_1), 7.48 (d, 1H, J = 8.9 Hz, H_4), 7.50 (m, 1H, $H_{4'}$), 7.55 (m, 2H, $H_{3'}$), 8.17 (m, 2H, $H_{2'}$), 9.27 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.71 (OCH_3), 104.22 (C-1), 111.92 (C-3′), 118.82 (C-4), 120.35 (C-1′), 120.10 (C-5′), 121.95 (C-3), 122.81 (C-8a), 131.02 (C-6′), 131.51 (C-4′), 138.21 (C-7), 145.73 (C-2), 146.36 (C-6), 147.79 (C-4a), 157.10 (C-2′), 172.41 (C-8).

4.1.6.2. 6-Amino-3-hydroxy-2-(2-methoxyphenyl)-4*H*-chromen-4-one (5b). White solid, yield: 98%, mol. wt: 283. Anal. Calcd for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.78; H, 4.65; N, 4.91. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.78 (s, 3H, $OCH_{3-2'}$), 5.45 (br s, 2H, NH_2), 7.05 (dd, 1H, J = 8.9, 2.5 Hz, H_3), 7.06 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.16 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.18 (d, 1H, J = 2.5 Hz, H_1), 7.33 (d, 1H, J = 8.9 Hz, H_4), 7.49 (t, 1H, J = 8.2 Hz, $H_{4'}$), 7.44 (d, 1H, J = 8.2 Hz, $H_{6'}$), 8.54 (br s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 112.63 (C-1), 113.29 (C-2′), 115.06 (C-4′), 119.34 (C-6′), 119.95 (C-4), 121.96 (C-8a), 125.71 (C-3), 129.64 (C-5′), 131.58 (C-2), 132.78 (C-1′), 138.60 (C-7), 144.46 (C-6), 148.72 (C-4a), 159.16 (C-3′), 172.58 (C-8).

4.1.6.3. 6-Amino-3-hydroxy-2-(3-methoxyphenyl)-4-oxo-4*H*-chromen-4-one (5c). White solid, yield: 98%, mol. wt: 283, mp: 152.7 °C. Anal. Calcd for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.81; H, 4.71; N, 4.87. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.09 (br d, 1H, J = 8.6 Hz, $H_{4'}$), 7.47 (t, 1H, J = 8.6 Hz, $H_{5'}$), 7.50 (d, 1H, J = 9.0 Hz, H_4), 7.75 (t, 1H, J = 1.8 Hz, $H_{2'}$), 7.77 (br d, 1H, J = 8.6 Hz, $H_{6'}$), 7.15 (dd, 1H, J = 9.0, 2.5 Hz, H_3), 7.05 (d, 1H, J = 2.5 Hz, H_1). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.36 (OCH_3), 104.21 (C-1), 114.01 (C-3′), 118.79, (C-4), 122.06 (C-3, C-8a), 124.05 (C-1′), 129.22 (C-2′), 137.41 (C-7), 144.89 (C-2), 145.82, (C-6), 147.19 (C-4a), 160.24 (C-4′), 172.38 (C-8).

4.1.6.4. 6-Amino-3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (5d). White solid, yield: 97%, mol. wt: 283. Anal. Calcd for $C_{16}H_{13}NO_4$: C, 67.84; H, 4.63; N, 4.94. Found: C, 67.83; H, 4.68; N, 4.89. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.83 (s, 3H, OCH_3), 5.44 (s, NH_2), 7.06 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.10 (d, 2H, J = 8.9 Hz, $H_{3'}$) 7.12 (d, 1H, J = 2.6 Hz, H_1), 7.45 (d, 1H, J = 8.9 Hz, H_4), 8.15 (d, 2H, J = 8.9 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.36 (OCH_3), 104.21 (C-1), 114.01 (C-3′), 118.79 (C-4), 122.06 (C-3, C-8a), 124.05 (C-1′), 129.22 (C-2′), 137.41 (C-7), 144.89 (C-2), 145.82 (C-6), 147.19 (C-4a), 160.24 (C-4′), 172.38 (C-8).

4.1.6.5. 6-Amino-2-(2-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (5e). White solid, yield: 98%, mol. wt: 271, mp: 198.9 °C. Anal. Calcd for $C_{15}H_{10}FNO_3$: C, 66.42; H, 3.72; N, 5.16. Found: C, 66.38; H, 3.74; N, 5.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 5.48 (s, 2H, NH_2), 7.07 (dd, 1H, J = 2.7, 8.8 Hz, H_3), 7.16 (d, 1H, J = 2.7 Hz, H_1), 7.35 (m, 2H, $H_{5',3'}$), 7.39 (d, 1H, J = 8.8 Hz, H_4), 7.58 (m, 1H, $H_{4'}$), 7.73 (td, 1H, J = 2.2, 7.6 Hz, $H_{6'}$), 8.72 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 104.14 (C-1), 116.96–116.25 (d, J = 21.8 Hz, C-3′), 118.85 (C-4), 119.30–119.41 (d, J = 14.3 Hz, C-1′), 122.27 (C-3), 122.70 (C-8a), 124.36–124.38 (d, J = 3.5 Hz, C-5′), 131.19 (C-6′), 132.17–132.28 (d, J = 8.6 Hz, C-4′), 138.57 (C-7), 142.76 (C-6), 145.95 (C-2), 147.75 (C-4a), 157.49–160.82 (d, J = 251.00 Hz, C-2′), 172.41 (C-8).

4.1.6.6. 6-Amino-2-(3-fluorophenyl)-3-hydroxy-4*H*-chromen-4-one (5f). White solid, yield: 96%, mol. wt: 271. Anal. Calcd for $C_{15}H_{10}FNO_3$: C, 66.42; H, 3.72; N, 5.16. Found: C, 66.39; H, 3.73; N, 5.11. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.10 (dd, 1H, J = 9.0, 2.7 Hz, H_3), 7.15 (d, 1H, J = 2.7 Hz, H_1), 7.32 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.50 (d, 1H, J = 9.0 Hz, H_4), 7.59 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.98 (dt, 1H, J = 11.1, 1.8 Hz, $H_{2'}$), 8.04 (br d, 1H, J = 8.0 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 103.93 (C-1),

113.83–114.15 (d, J = 24.1 Hz, C-2′), 116.14–116.41 (d, J = 20.4 Hz, C-4′), 118.96 (C-4), 122.06 (C-8a), 122.54 (C-3), 123.40 (C-6′), 130.50–130.61 (d, J = 8.3 Hz, C-5′), 133.88–133.99 (d, J = 8.3 Hz, C-1′), 138.71 (C-7), 142.80 (C-6), 145.96 (C-2), 147.26 (C-4a), 160.41–163.63 (d, J = 243 Hz, C-3′), 172.71 (C-8).

4.1.6.7. 6-Amino-2-(4-fluorophenyl)-3-hydroxy-4-oxo-4*H*-chromen-4-one (5g). Brown solid, yield: 96%, mol. wt: 271. Anal. Calcd for $C_{15}H_{10}FNO_3$: C, 66.42; H, 3.72; N, 5.16. Found: C, 66.41; H, 3.63; N, 5.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.42 (t, 2H, J = 8.9 Hz, $H_{3'}$), 7.60 (dd, 1H, J = 9.0, 2.4 Hz, H_3), 7.88 (d, 1H, J = 2.4 Hz, H_1), 7.84 (d, 1H, J = 9.0 Hz, H_4), 8.28 (dd, 2H, J = 8.9, 5.4 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 115.70 (C-1), 115.56–115;85 (d, J = 22.0 Hz, C-3′), 120.23 (C-4), 121.86 (C-8a), 127.24 (C-3), 127.70–127.74 (d, J = 2.5 Hz, C-1′), 130.21–130.30 (d, J = 8.0 Hz, C-2′), 132.48 (C-2), 138.80 (C-7), 144.85 (C-6), 152.09 (C-4a), 161.08–164.38 (d, J = 249.00 Hz, C-4′), 172.43 (C-8).

4.1.6.8. 6-Amino-3-hydroxy-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (5h). Pale brown solid, yield: 94%, mol. wt: 321. Anal. Calcd for $C_{16}H_{10}F_3NO_3$: C, 59.82; H, 3.14; N, 4.36. Found: C, 59.76; H, 3.16; N, 4.34. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.27 (dd, 1H, J = 2.8, 9.1 Hz, H_3), 7.39 (d, 1H, J = 2.8 Hz, H_1), 7.63 (d, 1H, J = 9.1 Hz, H_4), 7.80 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.85 (d, 1H, J = 8.2 Hz, $H_{4'}$), 8.45 (d, 1H, J = 8.2 Hz, $H_{6'}$), 8.52 (br s, 1H, $H_{2'}$), 9.75 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 108.11 (C-1), 119.67 (C-4), 122.21 (C-8a), 123.42 (q, J = 270.00 Hz, CF_3), 124.07 (q, J = 3.8 Hz, C-2′), 124.42 (C-3), 126.13 (q, J = 3.8 Hz, C-4′), 129.51 (q, J = 31.7 Hz, C-3′), 130.00 (C-5′), 131.27 (C-6′), 132.81 (C-1′), 139.35 (C-7), 141.61 (C-2), 143.09 (C-6), 149.14 (C-4a), 172.88 (C-8).

4.1.6.9. 6-Amino-3-hydroxy-4-oxo-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (5i). Pale brown solid, yield: 95%, mol. wt: 321. Anal. Calcd for $C_{16}H_{10}F_3NO_3$: C, 59.82; H, 3.14; N, 4.36. Found: C, 59.83; H, 3.18; N, 4.28. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.49 (dd, 1H, J = 2.6 Hz, 9.1 Hz, H_3), 7.71 (d, 1H, J = 2.6 Hz, H_1), 7.76 (d, 1H, J = 9.1 Hz, H_4), 7.93 (d, 2H, J = 8.5 Hz, $H_{3'}$), 8.41 (d, 2H, J = 8.5 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 113.08 (C-1), 120.17 (C-4), 122.08 (C-8a), 124.81 (q, J = 272 Hz, CF_3), 125.70 (q, J = 3.8 Hz, C-3′), 126.58 (C-3), 128.39 (C-2′), 129.50 (q, J = 32.4 Hz, C-4′), 135.48 (C-1′; C-2), 140.04 (C-7), 143.58 (C-6), 151.22 (C-4a), 172.91 (C-8).

4.1.7. General procedure for methylation of compounds (6a–i)

Flavonol **2a–i** (10 mmol) was solubilized in acetone (20 mL) with K_2CO_3 (1 equiv), then dimethyl sulfate (1 equiv) was introduced and the solution was heated at 50 °C for 6 h. The reaction was monitored by TLC and when no more beginning product appeared, the solvent was removed under reduce pressure. The mixture obtained was poured onto water (100 mL) and the pH was adjusted to 7. The precipitate obtained was filtered off and washed with water and dried to afford methoxyflavones **6a–i**.

4.1.7.1. *N*-(3-Methoxy-4-oxo-2-phenyl-4*H*-chromen-6-yl)acetamide (6a). Yellow solid, yield: 86%, mol. wt: 309. Anal. Calcd for $C_{18}H_{15}NO_4$: C, 69.89; H, 4.89; N, 4.53. Found: C, 69.83; H, 4.92; N, 4.49. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.56 (m, 2H, $H_{3'}$), 7.58 (m, 1H, $H_{4'}$), 7.67 (d, 1H, J = 9.1 Hz, H_4), 7.94 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.02 (m, 2H, $H_{2'}$), 8.37 (d, 1H, J = 2.5 Hz, H_1), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.01 (CH_3), 59.66 (OCH_3), 112.85 (C-1), 118.89 (C-4), 123.69 (C-8a), 125.56 (C-3), 128.20 (C-2′), 128.63 (C-3′), 130.52 (C-1′), 130.78 (C-4′), 136.39 (C-2), 140.42 (C-6), 150.62 (C-4a), 154.79 (C-7), 168.55 (CO), 173.70 (C-8).

4.1.7.2. *N*-(3-Methoxy-2-(2-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (6b). White solid, yield: 88%, mol. wt: 339. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.22; H, 5.12; N, 4.09. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 2H, CH_3), 3.71 (s, 3H, OCH_{3-7}), 3.81 (s, 3H, $OCH_{3-2'}$), 7.09 (t, 1H, J = 8.2 Hz, $H_{5'}$), 7.19 (d, 1H, J = 8.2 Hz, $H_{3'}$), 7.54 (t, 1H, J = 8.2 Hz, $H_{4'}$), 7.49 (d, 1H, J = 8.2 Hz, $H_{6'}$), 7.59 (d, 1H, J = 9.1 Hz, H_4), 7.92 (dd, 1H, J = 9.1, 2.6 Hz, H_3), 8.42 (d, 1H, J = 2.6 Hz, H_1), 10.29 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.01 (CH_3), 55.68 ($OCH_{3-2'}$), 59.66 (OCH_{3-8}), 111.74 (C-3′), 112.92 (C-1), 118.92 (C-4), 119.70 (C-1′), 120.25 (C-5′), 124.08 (C-8a), 125.47 (C-3), 130.44 (C-6′), 132.08 (C-4′), 136.37 (C-2), 140.85 (C-6), 151.04 (C-4a), 155.67 (C-7), 156.89 (C-2′), 168.60 (CO), 173.39 (C-8).

4.1.7.3. *N*-(3-Methoxy-2-(3-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (6c). Pale yellow solid, yield: 84%, mol. wt: 339. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.24; H, 5.11; N, 4.11. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 3H, CH_3), 3.82 (s, 3H, $OCH_{3-3'}$), 3.85 (s, 3H, OCH_{3-7}), 7.16 (dd, 1H, J = 8.0, 2.0 Hz, $H_{4'}$), 7.50 (t, 1H, J = 8.0 Hz, $H_{5'}$), 7.57 (t, 1H, J = 2.0 Hz, $H_{2'}$), 7.62 (br d, 1H, J = 8.0 Hz, $H_{6'}$), 7.72 (d, 1H, J = 9.1 Hz, H_4), 7.94 (dd, 1H, J = 9.1, 2.5 Hz, H_3), 8.38 (d, 1H, J = 2.5 Hz, H_1), 10.27 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.98 (CH_3), 55.29 ($OCH_{3-3'}$), 55.29 (OCH_{3-7}), 112.83 (C-1), 113.78 (C-2′), 116.33 (C-4′), 119.00 (C-4), 120.52 (C-6′), 123.67 (C-8a), 125.60 (C-3), 129.83 (C-5′), 131.72 (C-1′), 136.40 (C-2), 154.57 (C-7), 140.53 (C-6), 150.61 (C-4a), 159.17 (C-3′), 168.57 (CO), 173.71 (C-8).

4.1.7.4. *N*-(3-Methoxy-2-(4-methoxyphenyl)-4-oxo-4*H*-chromen-6-yl)acetamide (6d). White solid, yield: 91%, mol. wt: 339. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.21; H, 5.08; N, 4.08. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, CH_3), 3.79 (s, OCH_{3-7}), 3.86 (s, $OCH_{3-4'}$), 7.14 (d, 2H, J = 8.8 Hz, $H_{3'}$), 7.70 (d, 1H, J = 9.0 Hz, H_4), 7.93 (dd, 1H, J = 9.0, 2.3 Hz, H_3), 8.05 (d, 2H, J = 8.8 Hz, $H_{2'}$), 8.36 (d, 1H, J = 2.3 Hz, H_1), 10.29 (s, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.98 (CH_3), 55.41 ($OCH_{3-4'}$), 59.45 (OCH_{3-7}), 112.89 (C-1), 114.17 (C-3′), 118.81 (C-4), 122.64 (C-1′), 123.66 (C-8a), 125.40 (C-3), 130.00 (C-2′), 136.30 (C-2), 139.75 (C-6), 150.49 (C-4a), 154.79 (C-7), 161.17 (C-4′), 168.85 (CO), 173.47 (C-8).

4.1.7.5. *N*-(2-(2-Fluorophenyl)-3-methoxy-4-oxo-4*H*-chromen-6-yl)acetamide (6e). Yellow solid, yield: 82%, mol. wt: 327. Anal. Calcd for $C_{18}H_{14}FNO_4$: C, 66.05; H, 4.31; N, 4.28. Found: C, 65.98; H, 4.33; N, 4.23. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 7.40 (m, 2H, $H_{3',5'}$), 7.63 (m, 1H, $H_{4'}$), 7.64 (d, 1H, J = 8.8 Hz, H_4), 7.73 (td, 1H, J = 2.2, 7.6 Hz, $H_{6'}$), 7.94 (dd, 1H, J = 2.7, 8.8 Hz, H_3), 8.42 (d, 1H, J = 2.7 Hz, H_1), 10.29 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.16 (CH_3), 60.06 (OCH_{3-7}), 113.08 (C-1), 116.14–116.42 (d, J = 21.1 Hz, C-3′), 118.68–118.87 (d, J = 14.3 Hz, C-1′), 119.12 (C-4), 124.16 (C-8a), 124.84 (C-5′), 125.88 (C-3), 131.27 (C-6′), 133.12–133.23 (d, J = 6.8 Hz, C-4′), 136.71 (C-2), 141.19 (C-6), 151.14 (C-4a), 152.09 (C-7), 157.69–161.00 (d, J = 249.00 Hz, C-2′), 168.79 (CO), 173.51 (C-8).

4.1.7.6. *N*-(2-(3-Fluorophenyl)-3-methoxy-4-oxo-4*H*-chromen-6-yl)acetamide (6f). Yellow solid, yield: 71%, mol. wt: 327. Anal. Calcd for $C_{18}H_{14}FNO_4$: C, 66.05; H, 4.31; N, 4.28. Found: C, 66.01; H, 4.37; N, 4.24. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.41 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.62 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.70 (d, 1H, J = 9.0 Hz, H_4), 7.83 (dt, 1H, J = 11.1, 1.8 Hz, $H_{2'}$), 7.89 (br d, 1H, J = 8.0 Hz, $H_{6'}$), 7.93 (dd, 1H, J = 9.0, 2.5 Hz, H_3) 8.36 (d, 1H, J = 2.5 Hz, H_1). 10.26 (br s, 1H, NH).

^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.16 (CH_3), 59.89 (OCH_3), 112.98 (C-1), 114.95–115.26. (d, J = 23.4 Hz, C-2′), 117.72–118.00 (d, J = 21.1 Hz, C-4′), 119.20 (C-4), 123.83 (C-8a), 124.60–124.64 (d, J = 3.0 Hz, C-6′), 125.89 (C-3), 130.95–131.05 (d, J = 7.5 Hz, C-5′), 132.77–132.88 (d, J = 8.3 Hz, C-1′), 136.65 (C-2), 140.94 (C-6), 150.75 (C-4a), 153.25 (C-7), 160.51–163.74 (d, J = 243 Hz, C-3′), 168.78 (CO), 173.92 (C-8).

4.1.7.7. *N*-(2-(4-Fluorophenyl)-3-methoxy-4-oxo-4*H*-chromen-6-yl)acetamide (6g). White solid, yield: 86%, mol. wt: 327. Anal. Calcd for $C_{18}H_{14}FNO_4$: C, 66.05; H, 4.31; N, 4.28. Found: C, 66.07; H, 4.32; N, 4.21. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 7.09 (t, 2H, J = 9.0 Hz, $H_{3'}$), 7.37 (d, 1H, J = 9.2 Hz, H_4), 8.00 (d, 1H, J = 2.7 Hz, H_1), 8.01 (dd, 2H, J = 9.0, 5.4 Hz, $H_{2'}$), 8.24 (dd, 1H, J = 9.2, 2.7 Hz, H_3), 9.54 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 23.85 (CH_3), 59.61 (OCH_3), 113.96 (C-1), 115.17–115.46 (d, J = 21.9 Hz, C-3′), 118.02 (C-4), 123.74 (C-8a), 126.05 (C-3), 126.70 (C-1′), 130.32–130.44 (d, J = 9.0 Hz, C-2′), 136.10 (C-2), 140.43 (C-6), 150.91 (C-4a), 154.18 (C-7), 161.83–165.17 (d, J = 252 Hz, C-4′), 168.95 (CO), 174.32 (C-8).

4.1.7.8. *N*-(3-Methoxy-4-oxo-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (6h). White solid, yield: 85%, mol. wt: 377. Anal. Calcd for $C_{19}H_{14}F_3NO_4$: C, 60.48; H, 3.74; N, 3.71. Found: C, 60.45; H, 3.77; N, 3.68. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.72 (d, 1H, J = 9.2 Hz, H_4), 7.82 (t, 1H, J = 8.4 Hz, $H_{5'}$), 7.94 (br d, 1H, J = 8.4 Hz, $H_{4'}$), 7.94 (dd, 1H, J = 9.2, 2.5 Hz, H_3), 8.31 (br d, 1H, J = 8.4 Hz, $H_{6'}$), 8.32 (br s, 1H, $H_{2'}$), 8.37 (d, 1H, J = 2.5 Hz, H_1), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.10 (CH_3), 59.76 (OCH_3) 112.80 (C-1), 119.09 (C-4), 123.74 (C-8a), 123.42 (q, J = 270.00 Hz, CF_3), 124.69 (q, J = 3.8 Hz, C-2′), 125.73 (C-3), 127.22 (q, J = 3.8 Hz, C-4′), 129.47 (q, J = 32.4 Hz, C-3′), 129.97 (C-5′), 131.60 (C-6′), 132.22 (C-1′), 136.55 (C-2), 152.99 (C-7), 140.85 (C-6), 150.64 (C-4a), 168.63 (CO), 173.76 (C-8).

4.1.7.9. *N*-(3-Methoxy-4-oxo-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-6-yl)acetamide (6i). White solid, yield: 93%, mol. wt: 377. Anal. Calcd for $C_{19}H_{14}F_3NO_4$: C, 60.48; H, 3.74; N, 3.71. Found: C, 60.43; H, 3.75; N, 3.63. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.70 (d, 1H, J = 9.1 Hz, H_4), 7.94 (d, 2H, J = 8.9 Hz, $H_{3'}$), 7.95 (dd, 1H, J = 2.5, 9.1 Hz, H_3), 8.22 (d, 2H, J = 8.9 Hz, $H_{2'}$), 8.37 (d, 1H, J = 2.5 Hz, H_1), 10.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 24.19 (CH_3), 60.06 (OCH_{3-7}), 112.98 (C-1), 119.22 (C-4), 123.91 (C-8a), 124.81 (q, J = 272 Hz, CF_3), 125.72 (q, J = 3.8 Hz, C-3′), 126.01 (C-3), 129.29 (C-2′), 130.49 (q, J = 32.4 Hz, C-4′), 134.68 (C-1′), 136.73 (C-2), 141.27 (C-6), 150.83 (C-4a), 153.23 (C-7), 173.98 (C-8).

4.1.8. General procedure for deprotection of acetamido derivatives (7a–i)

Methoxyflavonol **5a–i** (10 mmol) was added to a solution of EtOH (20 mL) and H_2SO_4 concd (5 mL). The solution was submitted to MWI in open vessel 80 °C, 2 + 20 min, upon cooling the solvent was removed under vacuo and the residue obtain is poured onto ice water (100 mL). Then the solution was neutralized with NH_4OH 16% until pH 7. The red precipitate formed was collected by filtration and washed with excess cold water. Amino compound was obtained as a red powder without further purification.

4.1.8.1. 6-Amino-3-methoxy-2-phenyl-4*H*-chromen-4-one (7a). Yellow solid, yield: 80%, mol. wt: 267. Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.88; H, 4.92; N, 5.21. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.78 (s, 3H, OCH_3), 7.13 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.21 (d, 1H, J = 2.6 Hz, H_1), 7.48 (d, 1H, J = 8.9 Hz, H_4), 7.57 (m, 1H, $H_{4'}$), 7.58 (m, 2H, $H_{3'}$), 8.00 (m, 2H, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 59.61 (OCH_3), 105.55 (C-1), 119.00 (C-4), 122.56 (C-3), 124.46 (C-8a), 128.11 (C-2′), 128.62 (C-3′), 130.56 (C-1′), 130.85 (C-4′), 140.04 (C-6), 144.91 (C-2), 147.69 (C-4a), 154.33 (C-7) 173.62 (C-8).

4.1.8.2. 6-Amino-3-methoxy-2-(2-methoxyphenyl)-4*H*-chromen-4-one (7b). Brown solid, yield: 76%, mol. wt: 297. Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.66; H, 5.13; N, 4.68. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.74 (s, 3H, OCH_{3-7}), 3.81 (s, 3H, $OCH_{3-2'}$), 5.51 (s, 2H, NH_2), 7.01 (dd, 1H, J = 2.8, 8.9 Hz, H_3), 7.03 (t, 1H, J = 7.6 Hz, $H_{5'}$), 7.06 (d, 1H, J = 2.8 Hz, H_1), 7.28 (d, 1H, J = 8.9 Hz, $H_{3'}$), 7.42 (dd, 1H, J = 1.7, 7.6 Hz, $H_{6'}$), 7.44 (d, 1H, J = 2.8 Hz, H_4), 7.46 (t, 1H, J = 7.6 Hz, $H_{4'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 59.78 ($OCH_{3-2'}$), 60.13 (OCH_{3-7}), 107.06 (C-1), 111.22 (C-3′), 118.80 (C-4), 120.17 (C-5′), 120.25 (C-1′), 122.30 (C-3), 125.06 (C-8a), 130.39 (C-6′), 131.60 (C-4′), 141.06 (C-6), 143.93 (C-2), 149.25 (C-4a), 155.64 (C-7), 157.04 (C-2′), 174.48 (C-8).

4.1.8.3. 6-Amino-3-methoxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one (7c). Yellow solid, yield: 91%, mol. wt: 297. Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.61; H, 5.14; N, 4.69. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.77 (s, 3H, OCH_{3-7}), 3.82 ($OCH_{3-3'}$), 5.51 (s, 2H, NH_2), 7.07 (dd, 1H, J = 2.8, 8.9 Hz, H_3), 7.12 (dd, 1H, J = 2.0, 8.0 Hz, $H_{4'}$), 7.13 (d, 1H, J = 2.8 Hz, H_1), 7.46 (d, 1H, J = 8.9 Hz, H_4), 7.47 (t, 1H, J = 8.0 Hz, $H_{5'}$), 7.53 (t, 1H, J = 2.0 Hz, $H_{2'}$), 7.57 (d, 1H, J = 8.0 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.44 ($OCH_{3-3'}$), 59.78 (OCH_{3-7}), 104.55 (C-1), 113.84 (C-2′), 116.22 (C-4′), 119.13 (C-4), 120.58 (C-6′), 122.24 (C-3), 124.66 (C-8a), 129.95 (C-5′), 132.31 (C-1′), 140.27 (C-6), 146.50 (C-2), 147.32 (C-4a), 154.14 (C-7), 159.31 (C-3′), 173.85 (C-8).

4.1.8.4. 6-Amino-3-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (7d). Yellow solid, yield: 93%, mol. wt: 297. Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.09; N, 4.71. Found: C, 68.65; H, 5.09; N, 4.73. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.78 (s, OCH_{3-7}), 3.85 (s, $OCH_{3-4'}$), 7.13 (d, 2H, J = 8.8 Hz, $H_{3'}$), 7.27 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.40 (d, 1H, J = 2.6 Hz, H_1), 7.59 (d, 1H, J = 8.9 Hz, H_4), 8.03 (d, 2H, J = 8.8 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 55.43 ($OCH_{3-4'}$), 59.44 (OCH_{3-7}), 108.96 (C-1), 114.17 (C-3′), 119.31 (C-4), 122.83 (C-8a), 123.95 (C-3), 124.32 (C-1′), 129.94 (C-2′), 139.59 (C-6), 140.91 (C-2), 149.03 (C-4a), 154.58 (C-7), 161.10 (C-4′), 173.33 (C-8).

4.1.8.5. 6-Amino-2-(2-fluorophenyl)-3-methoxy-4*H*-chromen-4-one (7e). Yellow solid, yield: 82%, mol. wt: 285. Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; N, 4.91. Found: C, 67.29; H, 4.27; N, 4.89. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.78 (s, 3H, OCH_3), 7.19 (dd, 1H, J = 2.7, 8.8 Hz, H_3), 7.33 (d, 1H, J = 2.7 Hz, H_1), 7.42 (m, 1H, $H_{3'}$), 7.46 (d, 1H, J = 8.8 Hz, H_4), 7.64 (m, 1H, $H_{4'}$), 7.73 (td, 1H, J = 2.2, 7.6 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 53.89 (OCH_{3-7}), 107.19 (C-1), 116.11–116.39 (d, J = 21.1 Hz, C-3′), 118.93–119.36 (d, J = 14.3 Hz, C-1′), 119.19 (C-4), 123.43 (C-3), 124.58 (C-5′), 124.69 (C-8a), 131.03 (C-6′), 132.96–133.08 (d, J = 7.5 Hz, C-4′), 140.68 (C-6), 143.47 (C-2), 148.74 (C-4a), 151.58 (C-7), 160.99–162.48 (d, J = 250.00 Hz, C-2′), 173.20 (C-8).

4.1.8.6. 6-Amino-2-(3-fluorophenyl)-3-methoxy-4*H*-chromen-4-one (7f). Yellow solid, yield: 89%, mol. wt: 285. Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; N, 4.91. Found: C, 67.32; H, 4.31; N, 4.87. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.80 (OCH_3), 7.08 (dd, 1H, J = 8.9, 2.7 Hz, H_3), 7.12 (d, 1H, J = 2.7 Hz, H_1), 7.40 (td, 1H, J = 8.5, 2.3 Hz, $H_{4'}$), 7.48 (d, 1H, J = 8.9 Hz, H_4), 7.61 (td, 1H, J = 8.0, 6.3 Hz, $H_{5'}$), 7.81 (dt, 1H, J = 11.1, 1.8 Hz, $H_{2'}$), 7.87 (br d, 1H, J = 8.0 Hz, $H_{6'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 59.81 (OCH_3), 104.55 (C-1), 114.82–115.14 (d, J = 24.1 Hz, C-2′),

117.42–117.69 (d, J = 20.3 Hz, C-4′), 119.16 (C-4), 122.41 (C-3), 124.47–124.49 (d, J = 3.0 Hz, C-6′), 124.64 (C-8a), 130.88–130.98 (d, J = 7.5 Hz, C-5′), 133.17–133.28 (d, J = 8.3 Hz, C-1′), 140.48 (C-6), 146.45 (C-2), 147.32 (C-4a), 152.69 (C-7) 160.51–163.73 (d, J = 243 Hz, C-3′), 172.83 (C-8).

4.1.8.7. 6-Amino-2-(4-fluorophenyl)-3-methoxy-4*H*-chromen-4-one (7g). Yellow solid, yield: 91%, mol. wt: 285. Anal. Calcd for $C_{16}H_{12}FNO_3$: C, 67.36; H, 4.24; N, 4.91. Found: C, 67.35; H, 4.29; N, 4.90. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.77 (s, 3H, OCH_3), 5.51 (s, 2H, NH_2), 7.06 (dd, 1H, J = 2.8, 8.8 Hz, H_3), 7.12 (d, 1H, J = 2.8 Hz, H_1), 7.40 (d, 2H, J = 8.9, 5.3 Hz, $H_{3'}$), 7.44 (d, 1H, J = 8.8 Hz, H_4), 8.28 (d, 2H, J = 8.9 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 59.76 (OCH_{3-7}), 104.59 (C-1), 115.77–116.06 (d, J = 21.9 Hz, C-3′), 119.07 (C-4), 122.24 (C-3), 124.68 (C-8a), 127.58–127.60 (d, J = 3.0 Hz, C-1′), 130.84–130.96 (d, J = 9.0 Hz, C-2′), 140.00 (C-6), 146.54 (C-2), 147.30 (C-4a), 153.48 (C-7), 161.65–164.96 (d, J = 250.00 Hz, C-4′), 173.81 (C-8).

4.1.8.8. 6-Amino-3-methoxy-2-(3-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (7h). Brown solid, yield: 77%, mol. wt: 335. Anal. Calcd for $C_{17}H_{12}F_3NO_3$: C, 60.90; H, 3.61; N, 4.18. Found: C, 60.88; H, 3.65; N, 4.12. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.82 (s, 3H, OCH_3), 5.56 (br s, 2H, NH_2), 7.09 (dd, 1H, J = 8.9, 2.6 Hz, H_3), 7.14 (d, 1H, J = 2.6 Hz, H_1).7.51 (d, 1H, J = 8.9 Hz, H_4), 7.82 (t, 1H, J = 8.4 Hz, $H_{5'}$), 7.93 (br d, 1H, J = 8.4 Hz, $H_{4'}$), 8.30 (br d, 1H, J = 8.4 Hz, $H_{6'}$), 8.30 (br s, 1H, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 59.72 (OCH_3) 104.28 (C-1), 119.08 (C-4), 124.56 (C-8a), 123.42 (q, J = 270.00 Hz, CF_3), 124.55 (q, J = 3.8 Hz, C-2′), 122.25 (C-3), 126.97 (q, J = 3.8 Hz, C-4′), 129.41 (q, J = 32.4 Hz, C-3′), 129.65 (C-5′), 132.11 (C-6′), 131.97 (C-1′), 146.48 (C-2), 152.41 (C-7), 140.37 (C-6), 147.19 (C-4a), 173.68 (C-8).

4.1.8.9. 6-Amino-3-methoxy-2-(4-(trifluoromethyl)phenyl)-4*H*-chromen-4-one (7i). Yellow solid, yield: 86%, mol. wt: 335. Anal. Calcd for $C_{17}H_{12}F_3NO_3$: C, 60.90; H, 3.61; N, 4.18. Found: C, 60.86; H, 3.66; N, 4.14. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.80 (s, 3H, OCH_3), 5.54 (s, 2H, NH_2), 7.09 (dd, 1H, J = 2.5, 9.1 Hz, H_3), 7.12 (d, 1H, J = 2.5 Hz, H_1), 7.47 (d, 1H, J = 9.1 Hz, H_4), 7.92 (d, 2H, J = 8.9 Hz, $H_{3'}$), 8.21 (d, 2H, J = 8.9 Hz, $H_{2'}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm: 60.01 (OCH_{3-7}), 104.49 (C-1), 119.17 (C-4), 122.53 (C-3), 124.75 (C-8a), 124.81 (q, J = 272 Hz, CF_3), 125.69 (q, J = 3.8 Hz, C-3′), 129.16 (C-2′), 130.19 (q, J = 32.4 Hz, C-4′), 135.07 (C-1′), 140.81 (C-6), 146.67 (C-2), 147.69 (C-4a), 152.66 (C-7), 173.91 (C-8).

4.2. Material and methods

4.2.1. General

Cell culture medium (RPMI 1640), fetal calf serum (FCS), L-glutamine, non essential amino acids and others medium additives were from Eurobio (Paris, France). All other chemicals were of highest chemical purity and were purchased from Sigma except contrary mention. Stock solutions of quinazoline derivatives were prepared in DMSO. Stock solutions of reference drugs were prepared in ultrapure H_2O or DMSO. Flow cytometry was performed at the Faculté de Pharmacie de Marseille, using a FACSort flow cytometer apparatus (Beckton Dickinson, Paris, France), equipped with an argon laser (power of 15 mW, and wavelength of 488 nm).

4.2.2. Cytotoxic assays on human monocyte THP1

Cytotoxicity of chalcone and flavone derivatives was assessed on human monocyte THP1 (ATCC, Manassas VA, USA) by flow cytometry.[27] Briefly, late log-phase THP1 cells (10^5 cells/mL) were incubated in RPMI 1640 (without phenol red) supplemented with 10% FCS and 1% L-glutamine/penicillin–streptomycin mix with a range of compound concentrations incorporated in duplicate (final DMSO concentration less than 0.5%). Appropriate controls treated with or without solvent (DMSO), and various concentrations of doxorubicin and chloroquine were added to each set of experiments. After 72 h incubation at 37 °C and 6% CO_2, cells were stained with 5 μL of propidium iodide (1 mg/mL), and their growth and viability were measured by flow cytometry using a FACSort flow cytometer (Beckton Dickinson, Paris, France), equipped with an argon laser (power, 15 mW; wavelength, 488 nm). Settings were: Forward Scatter (FSC-H), size: Voltage E-1, gain 1, mode Log; Side Scatter (SSC-H), granulosity: Voltage 250, gain 1, mode Log; Fluorescence 2 (FL2), red fluorescence: Voltage 551, gain 1, mode Log.

IC_{50}THP1 was defined as the concentration of compound required to induce a 50% decrease of cell growth as compared to the control culture. It was calculated by non-linear regression analysis processed on dose–response curves by the Table Curve software 2D v.5.0. IC_{50} values represent the mean value calculated from three independent experiments.

4.2.3. Antiplasmodial activity

In this study, the W2 culture-adapted Plasmodium falciparum reference strain was used. It is resistant to chloroquine, pyrimethamine and proguanil. Maintenance in continuous culture was done as described previously by Trager and Jensen.[28] Parasites were cultivated in 75 cm^2-flasks containing RPMI 1640 (20 mL) supplemented with 25 mM HEPES, 25 mM $NaHCO_3$, 10% of A+ human serum and 1 mL of washed erythrocytes (final hematocrit 2.5%). Parasitaemia was maintained daily between 1% and 6%. Dilutions used non-infected A+ erythrocytes. Cultures were incubated at 37 °C, 10% O_2, 6% CO_2, 84% N_2, with 90% humidity. Cultures were monitored daily by microscopic examination of blood smears fixed with methanol and stained with 10% Giemsa stain. Parasite growth was assessed by flow cytometry according to a methodology previously described using hydroethidine (HE, Interchim, Montluçon, France) that is converted by metabolically active parasites into ethidium.[27] After incubation with hydroethidine, parasitized and uninfected erythrocytes were all identified on the basis of fluorescence intensity and size. Triplicate assays were performed in 96-well plates (Nunc Brand products, Fisher, Paris, France) containing 200 μL of asynchronous parasite cultures at 2% of parasitaemia and 2% hematocrit, and 5 μL of the appropriate extract dissolved in DMSO or ultrapure H_2O. Negative control, treated by solvents (DMSO or H_2O) and positive controls (chloroquine) were added to each set of experiments. After 48 h incubation without medium change, plates were centrifuged and the upper liquids were replaced with 200 μL hydroethidine solution (0.05 mg/mL in phosphate buffered saline (PBS)). Plates were incubated 20 min in the dark at 37 °C and washed three times with PBS. Finally, cells were suspended in 1 mL of PBS to allow determination of the number of cell events (around 300 per second) and parasitaemia by flow cytometry using a FACSort flow cytometer. Settings were: Forward Scatter (FSC-H), size: Voltage E-1, gain 1, mode Log; Side Scatter (SSC-H), granulosity: Voltage 250, gain 1, mode Log; Fluorescence 2 (FL2), red fluorescence: Voltage 459, gain 1, mode Log. The concentrations of compounds required to induce a 50% decrease of infected erythrocytes (IC_{50}W2) was calculated from three independent experiments.

4.2.4. Anti-HIV assays and cytotoxicity on MT-4 cells

The activity of the compounds against HIV-1 (III_B strain) and HIV-2 (ROD) was based on the inhibition of virus-induced cytopathic effect in MT-4 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[29] Briefly, virus stocks were titrated in MT-4 cells and expressed as 50% cell culture infective dose (CC_{50}). MT-4 cells were suspended in culture

medium at 10^5 cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after virus infection, 100 mL of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. The test compounds were dissolved in DMSO at 50 mM or higher. After a 4-day incubation at 37 °C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effect in uninfected MT-4 cells.

4.2.5. Selectivity index (SI)

The selectivity indexes that are presented correspond to the ratios between, respectively, the toxicity on THP1 or MT-4 cell lines and the W2 antiplasmodial activity or anti-HIV activity. They are calculated as follows: SI W2 = IC_{50} (THP1)/IC_{50} (W2), SI HIV-$1III_B$ = CC_{50} (MT-4)/IC_{50} (HIV-$1III_B$), and SI HIV-2ROD = CC_{50} (MT-4)/IC_{50} (HIV-2ROD).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.067. These data include MOL files and InChiKeys of the most important compounds described in this article.

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