Electronic Supplementary Material (ESI) for RSC Advances.

Mannich bases of hydroxycoumarins: synthesis, DFT/QTAIM computational study and assessment of biological activity.

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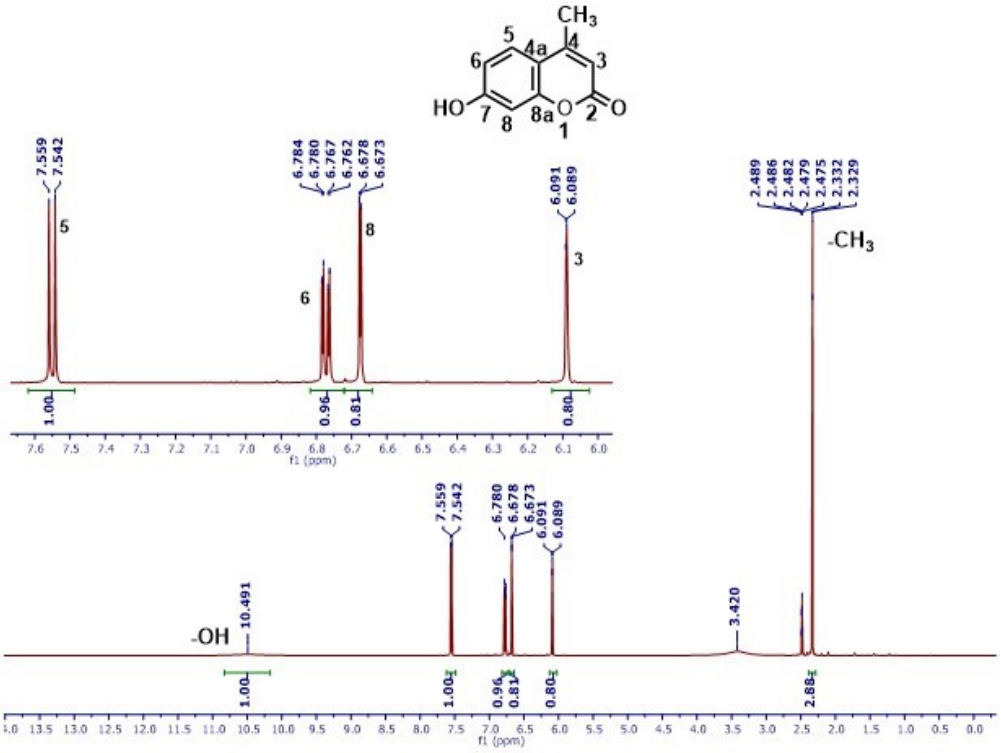
Supplementary Information (SI)

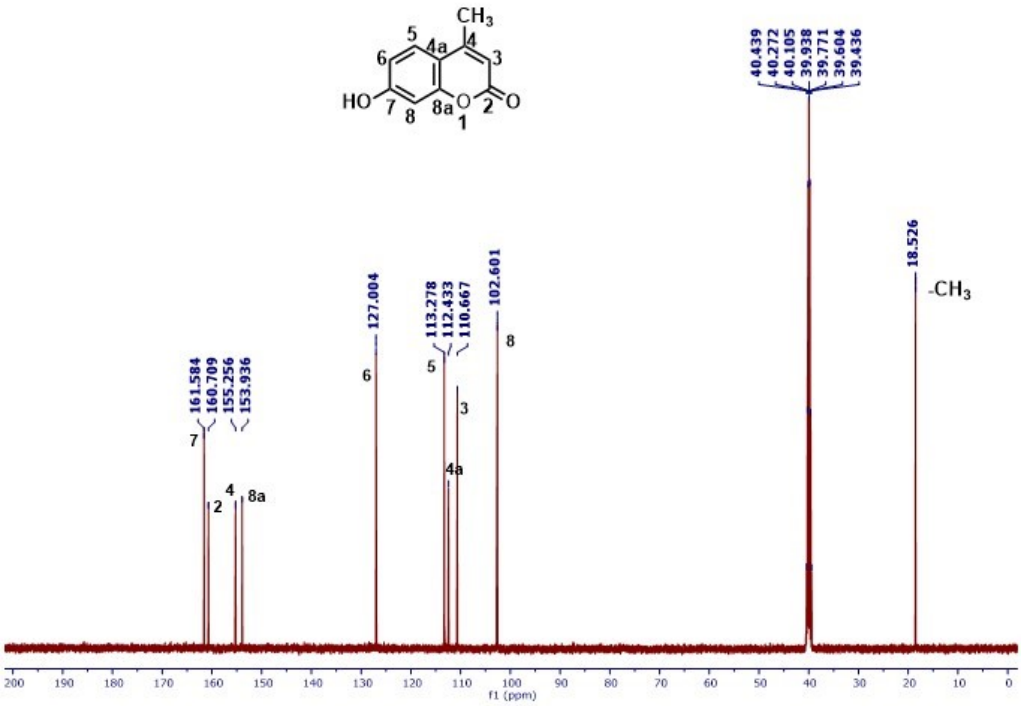
1. Synthesis and characterization

^{1}H-NMR spectroscopy of the derivatives of 7-hydroxycoumarin (Compounds **5a**-**5c**) shows the expected pattern of signals, two double signals in 7.50 and 6.70 ppm approximately, both with coupling constants near to 8.8 Hz and integrals to one hydrogen nuclei. These signals are the expected for the hydrogen atoms in positions 5 and 6 (see Scheme 1) of a tetrasubstituted aromatic ring. In 6.09 ppm the vinylic signal is found, and at low frequency the methylene and methyl substituents appear at 3.90 and 2.34 ppm, respectively. The signals of substituents of the secondary amines appear in the expected region of aliphatic groups, for instance in compound **5a** specifically as multiple signals in 2.53 ppm, which was assigned as de hydrogen atoms alpha to the nitrogen of the amine, and the rest of the chain in 1.54 and 1.43 ppm, respectively. The results of ^{1}H-NMR spectroscopy of the derivatives of 6,7-dihydroxycoumarin (Compounds **5d**-**5f**) shows the expected single signal around 6.89 ppm which was assigned to the only aromatic hydrogen nucleus in the benzene ring of the coumarin, in 5.99 the vinylic signal is observed, in most of the cases the long range coupling with the methyl in position 4 is observed, the rest of the molecule, both the methylene and the methyl signals and the aliphatic chain of the substituents of the amine group are observable within the aliphatic region of the spectra.

1.1 7-hydroxy-4-methylcoumarin (**4a**).

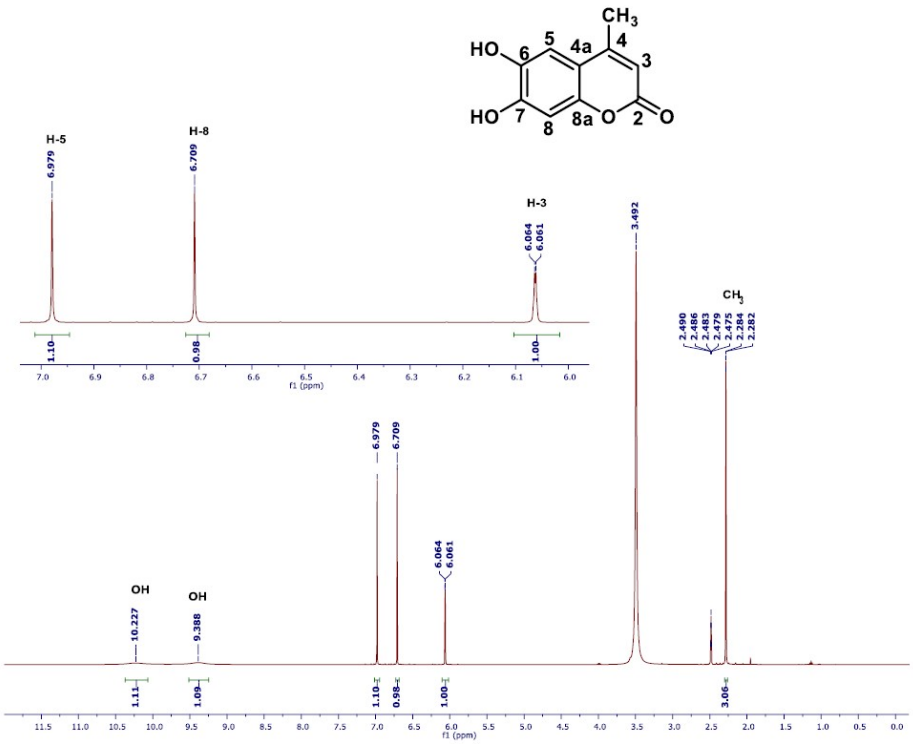


^{1}H-NMR (500 MHz, DMSO-d_6)

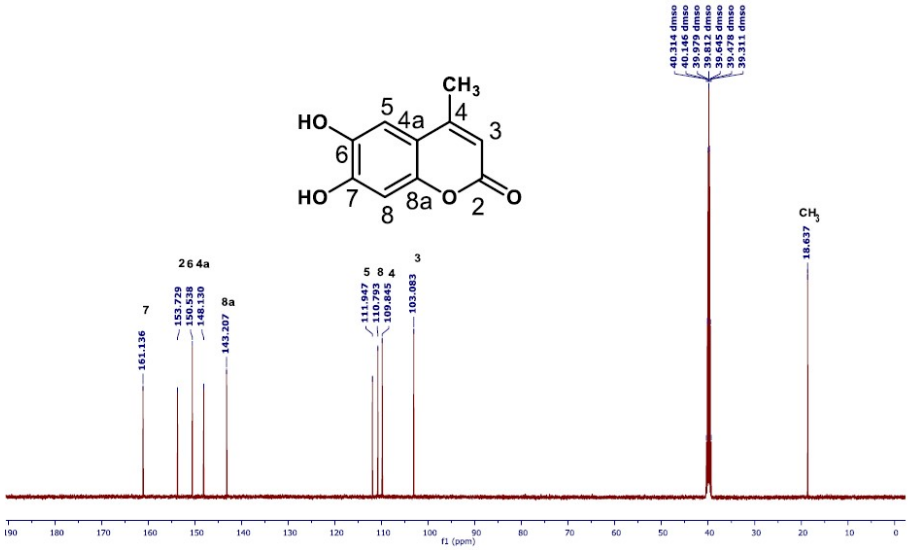


^{13}C-NMR (125 MHz, DMSO-d_6)

1.2 6,7-dihydroxy-4-methylcoumarin (**4b**).

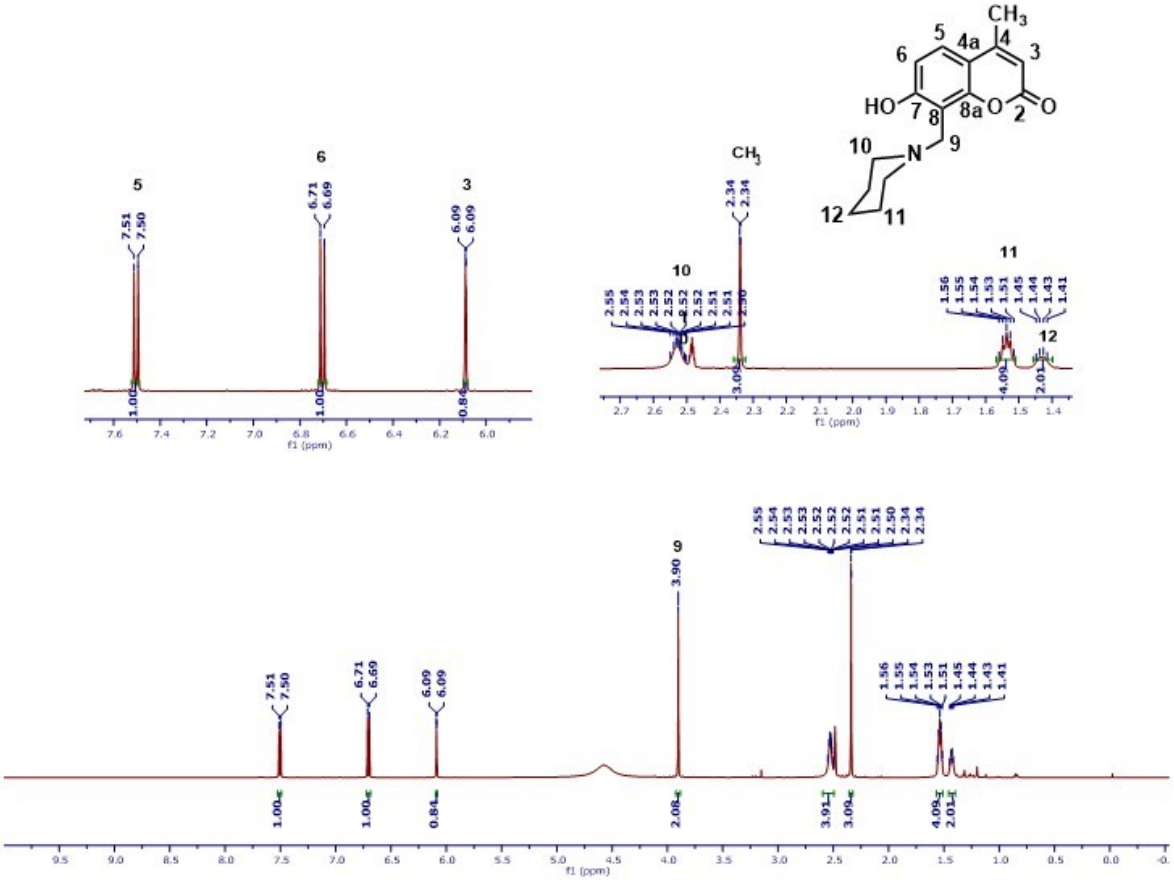


^{1}H-NMR (500 MHz, DMSO-d_6)

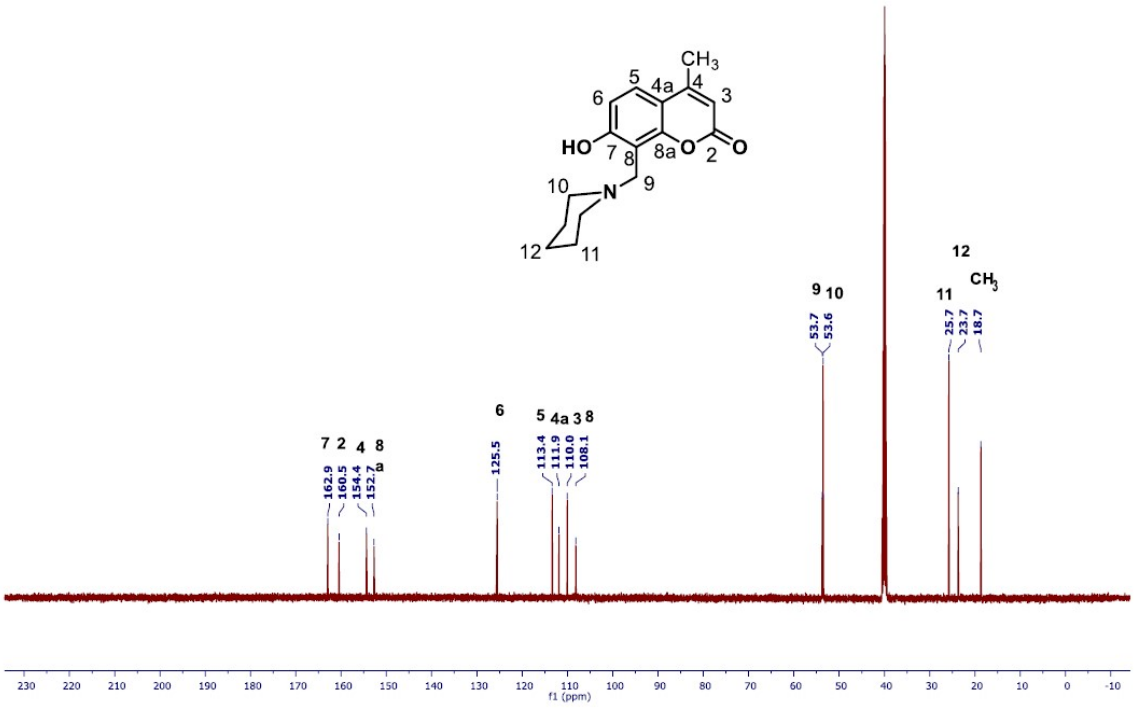


^{13}C-NMR (125 MHz, DMSO-d_6)

1.3 7-hydroxy-4-methyl-8-(piperidin-1-ylmethyl)-2H-chromen-2-one (**5a**).

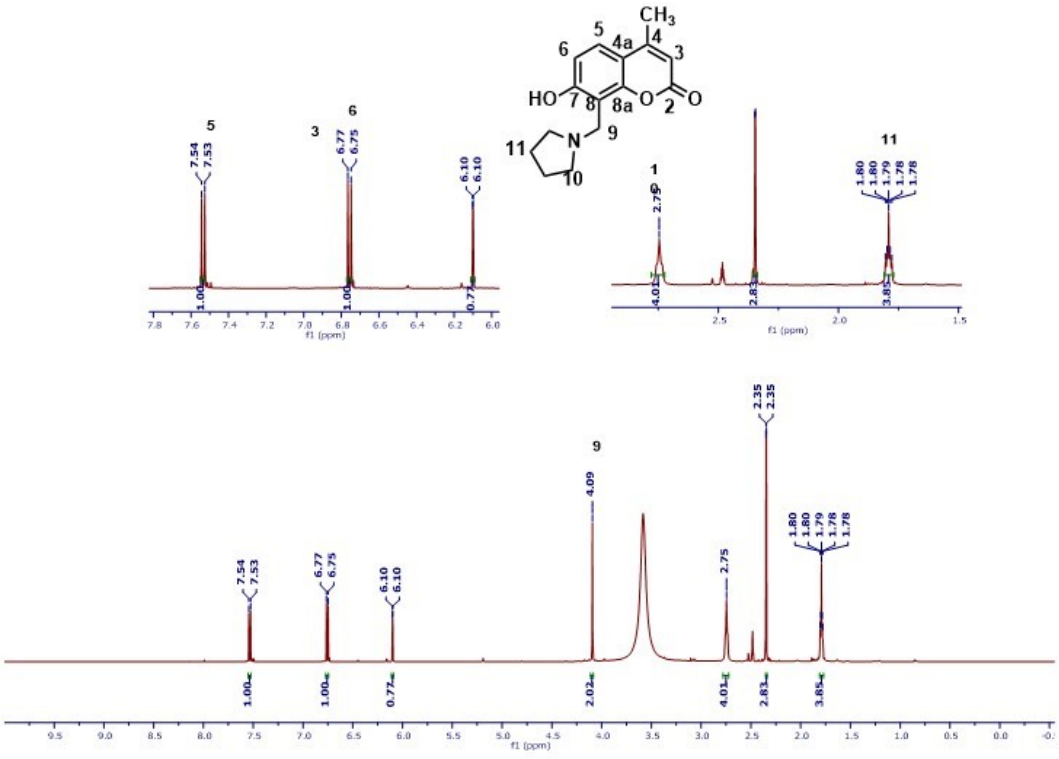


^{1}H-NMR (500 MHz, DMSO-d_6)

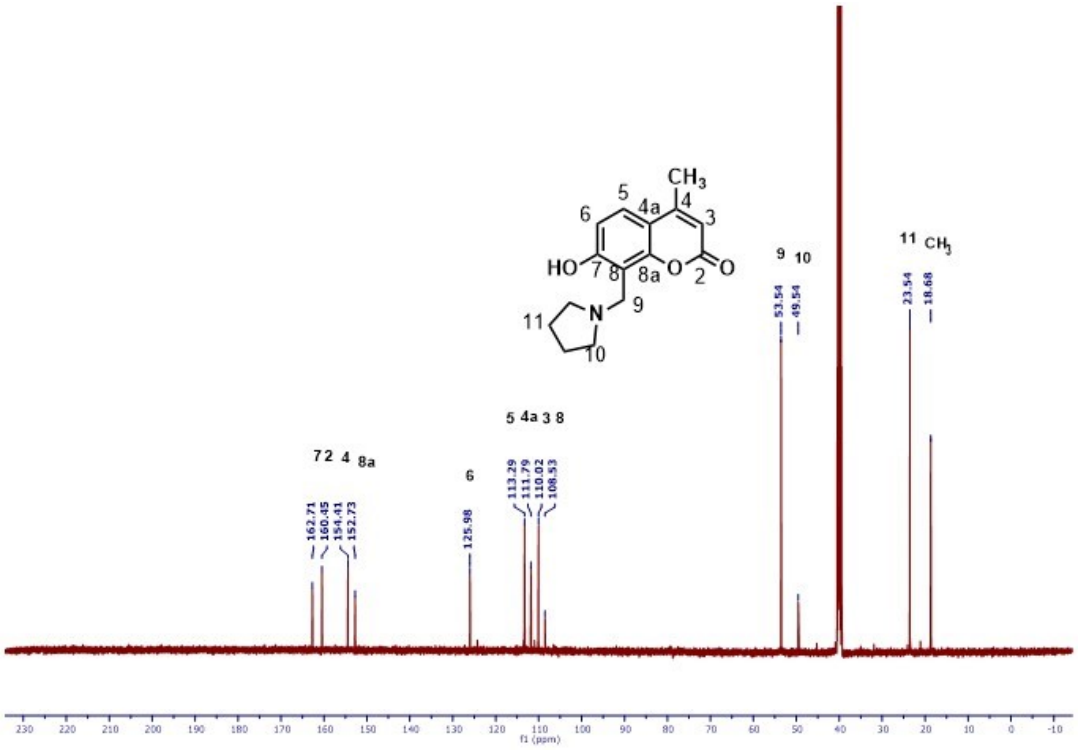


^{13}C-NMR (125 MHz, DMSO-d_6)

1.4 7-hydroxy-4-methyl-8-(pyrrolidin-1-ylmethyl)-2H-chromen-2-one (**5b**).

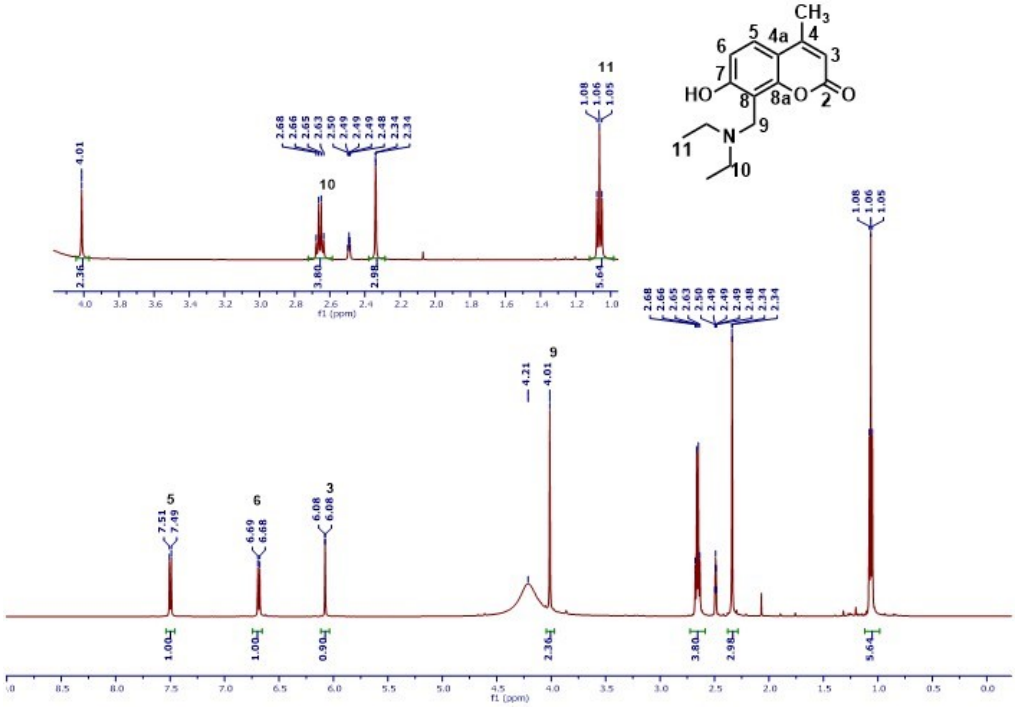


^{1}H-NMR (500 MHz, DMSO-d_6)

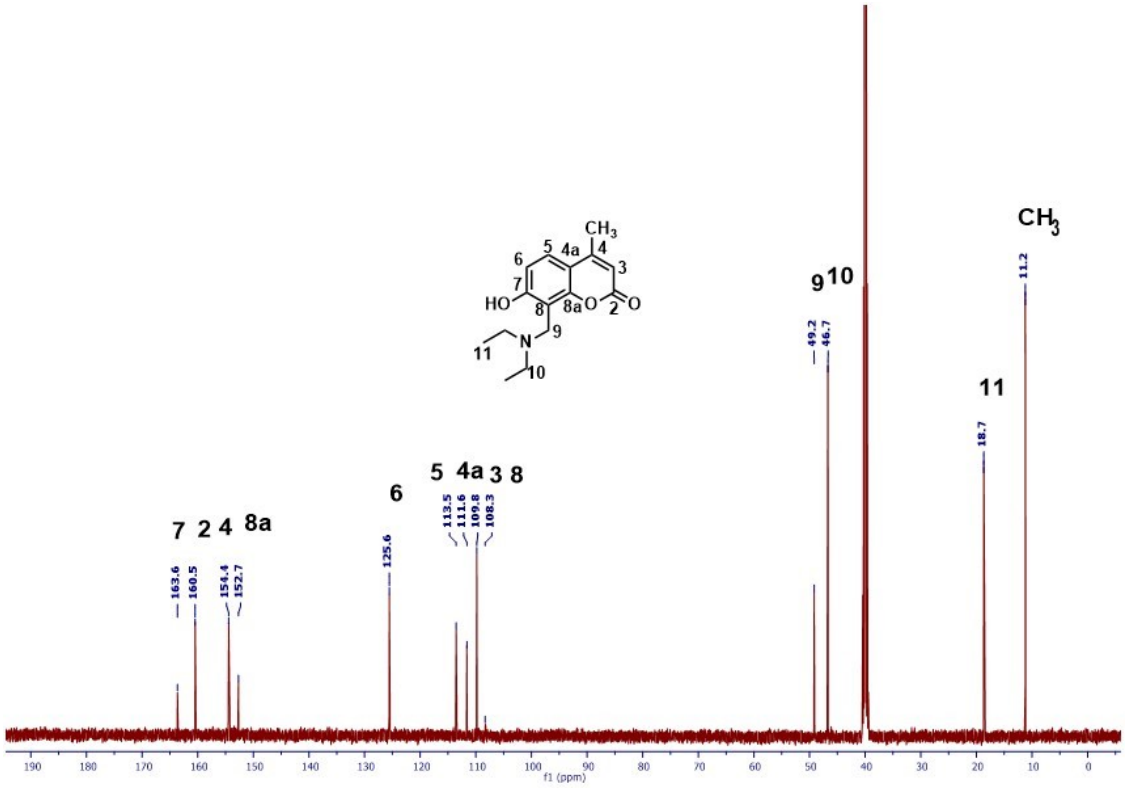


^{13}C-NMR (125 MHz, DMSO-d_6)

1.5 8-((diethylamino)methyl)-7-hydroxy-4-methyl-2H-chromen-2-one (**5c**).

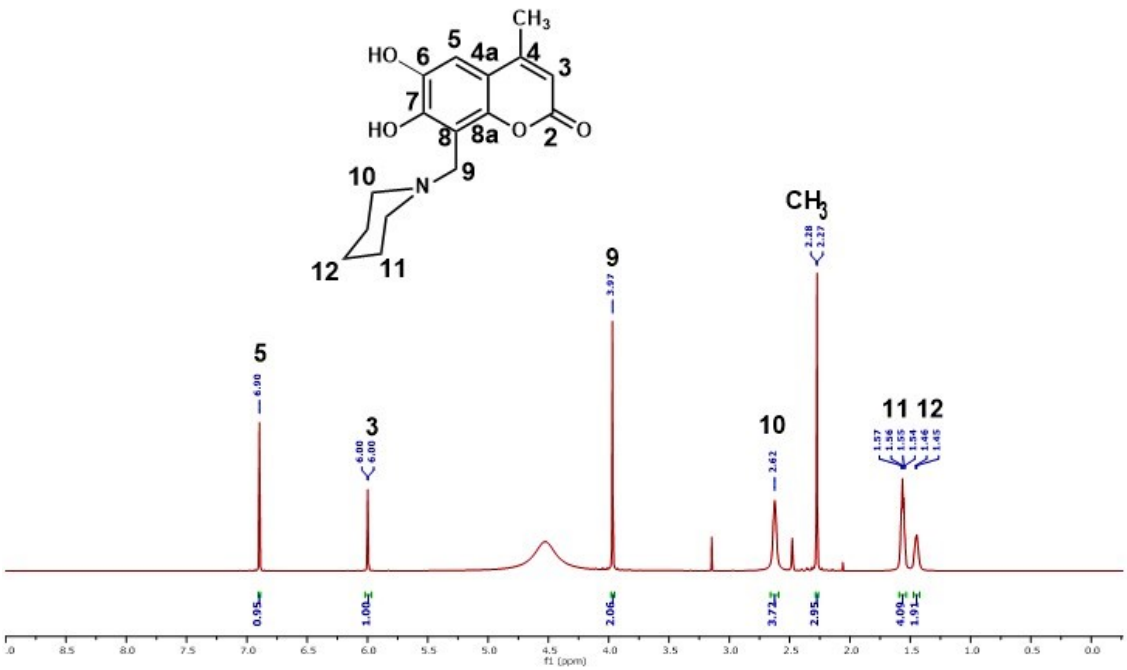


^{1}H-NMR (500 MHz, DMSO-d_6)

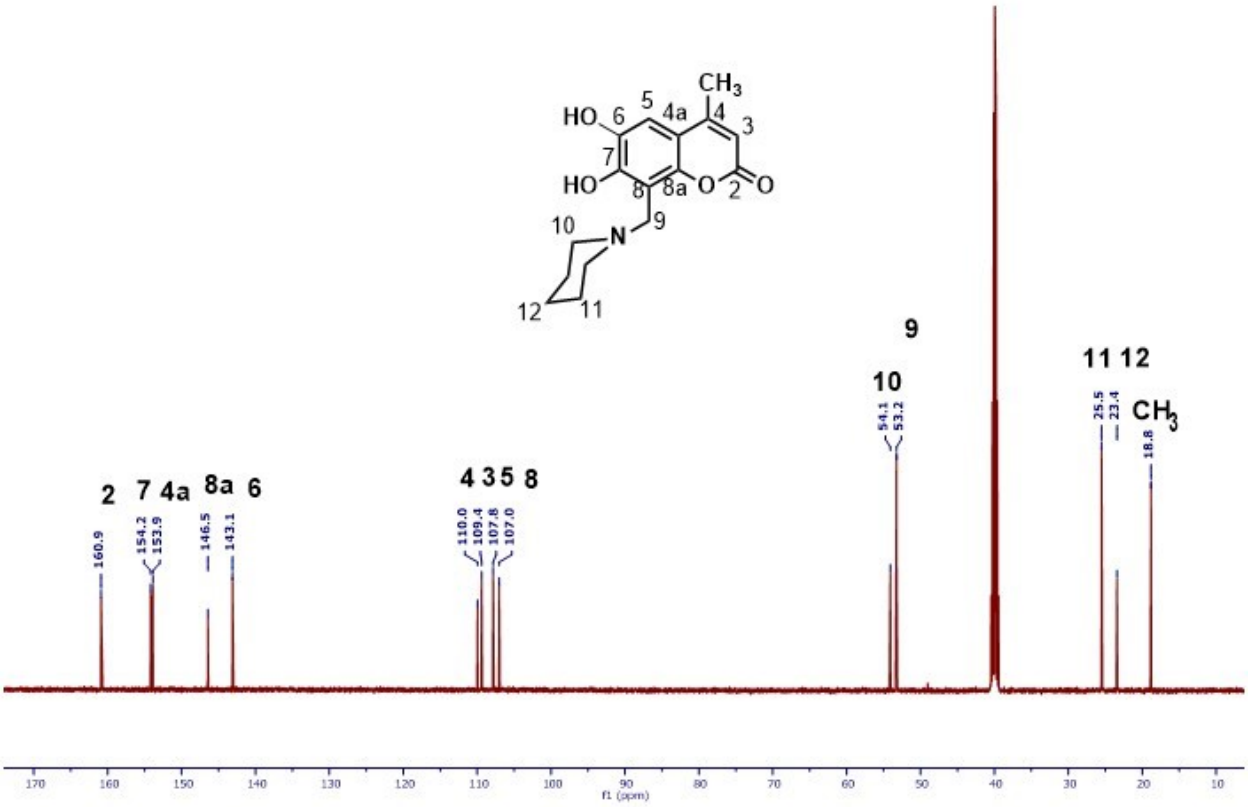


^{13}C-NMR (125 MHz, DMSO-d_6)

1.6 6,7-dihydroxy-4-methyl-8-(piperidin-1-ylmethyl)-2H-chromen-2-one (**5d**).

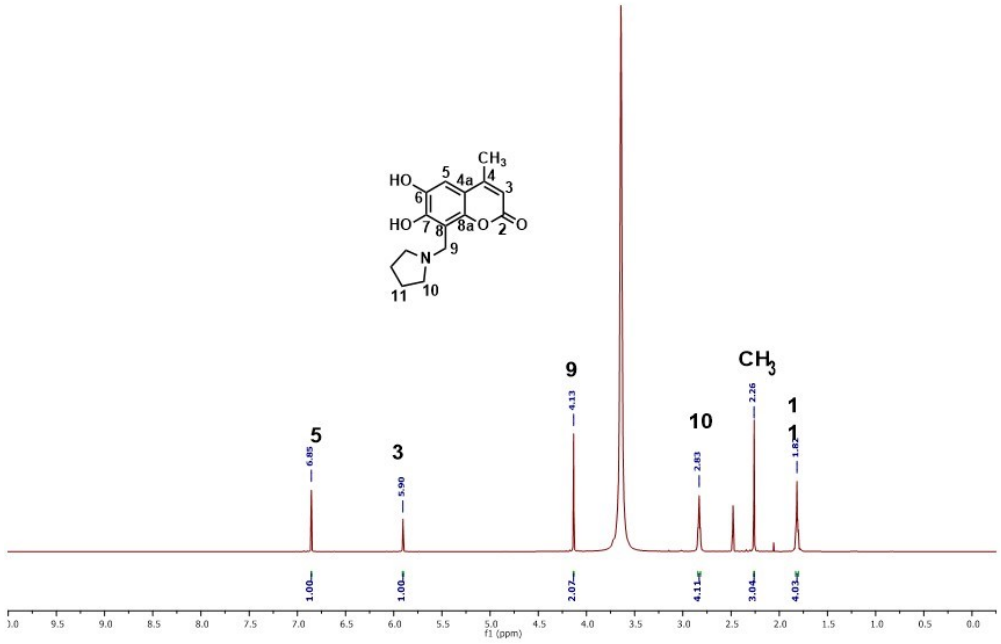


^{1}H-NMR (500 MHz, DMSO-d_6)

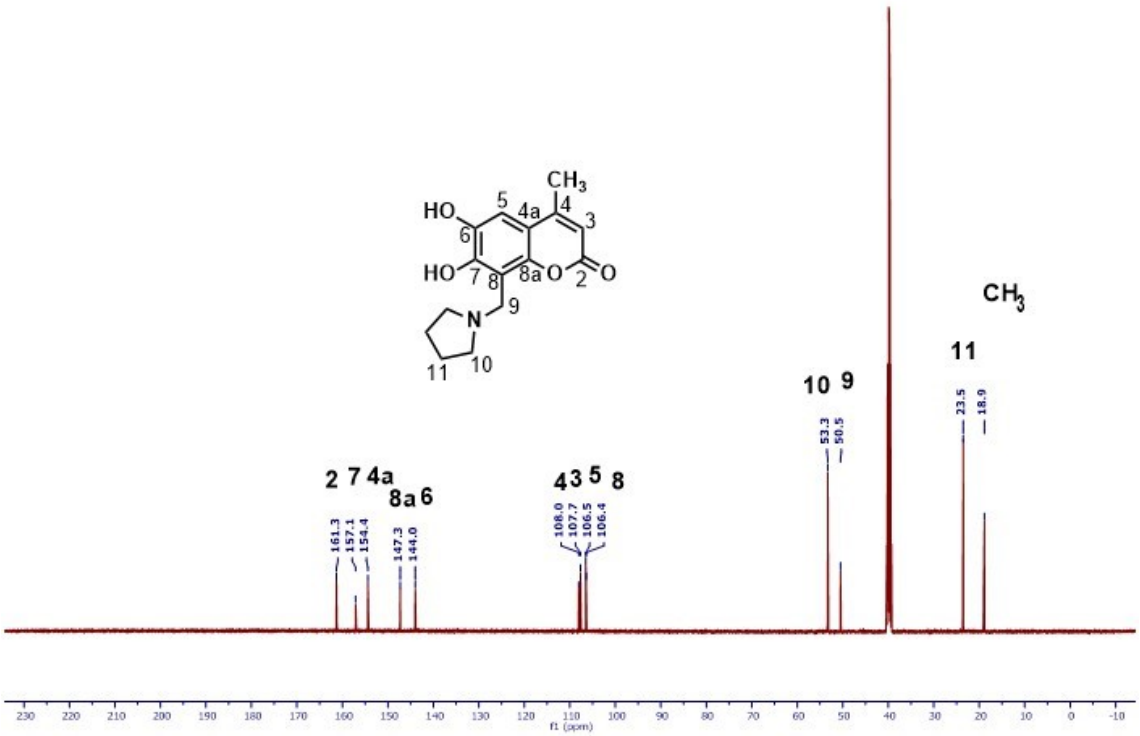


^{13}C-NMR (125 MHz, DMSO-d_6)

1.7 6,7-dihydroxy-4-methyl-8-(pyrrolidin-1-ylmethyl)-2H-chromen-2-one (**5e**).

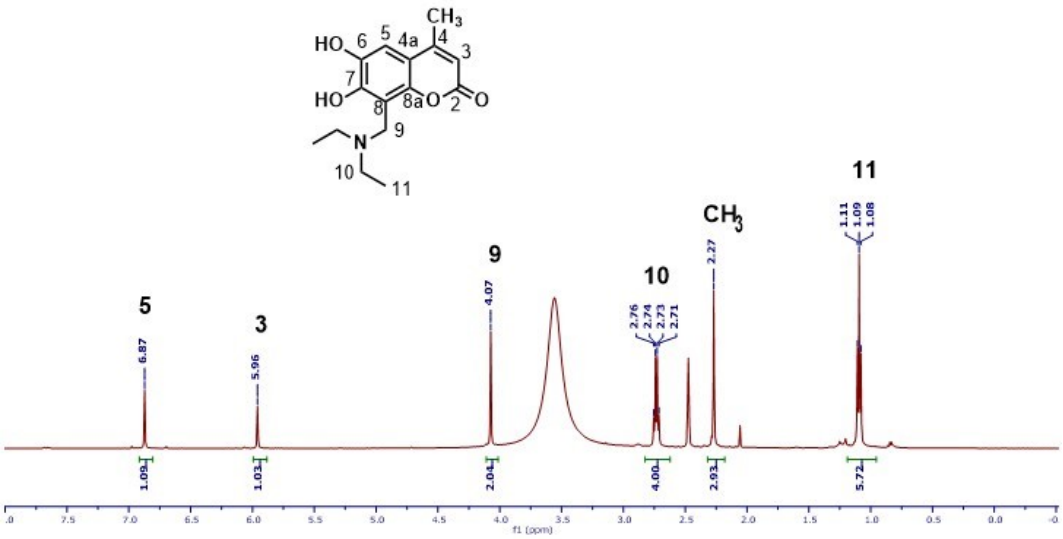


^{1}H-NMR (500 MHz, DMSO-d_6)

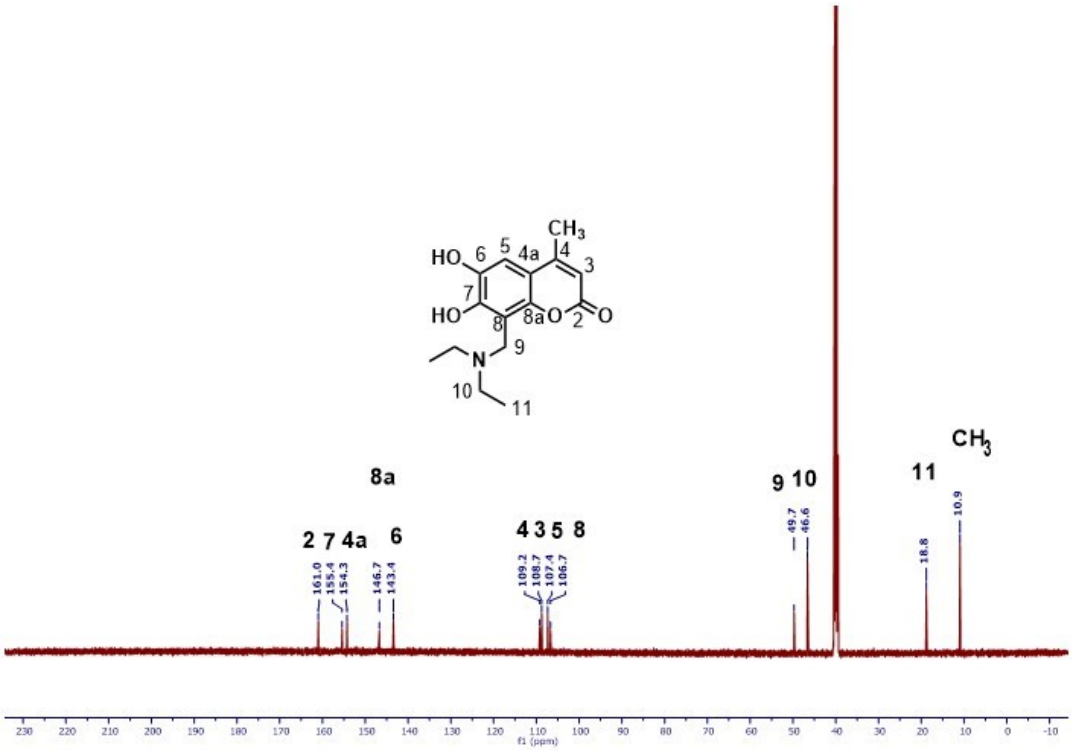


^{13}C-NMR (125 MHz, DMSO-d_6)

1.8 8-((diethylamino)methyl)-6,7-dihydroxy-4-methyl-2H-chromen-2-one (**5f**).

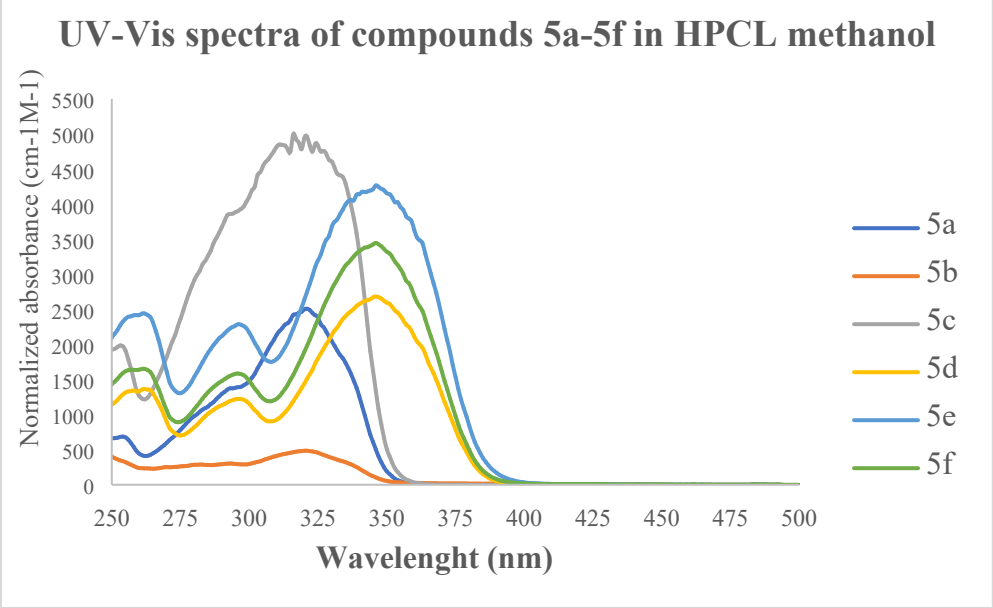


^{1}H-NMR (500 MHz, DMSO-d_6)



^{13}C-NMR (125 MHz, DMSO-d_6)

2. UV-Vis normalized absorption in HPLC Methanol



3. Computational details

3.1 Cartesian coordinates optimized of 7-hydroxy-4-methylcoumarin (**4a**) at PBE0/aug-cc-pvTZ level theory using the SMD model solvation.

Charge = 0 Multiplicity = 1

C	-2.34100691	1.03084609,	0.00000117
C	-2.52596093	-0.35588202	-0.00000176
C	-1.43798335	-1.21376906	-0.00000185
C	-0.16621321	-0.66869536	0.00000282
C	0.05902087	0.71070432	0.00000625
C	-1.06723719	1.54631007	0.00000413
O	0.86973748	-1.55336406	0.00000777
C	2.17501383	-1.13851704	0.00003134
C	2.42139208	0.26925156	0.00000313
C	1.41737450	1.18152874	0.00000216
O	3.02077520	-2.01841267	-0.00003363
C	1.70656545	2.64036808	-0.00000933
O	-3.80049169	-0.81283583	-0.00000560
H	-3.20735306	1.67973423	-0.00000014
H	-1.57166077	-2.28869372	-0.00000402
H	-0.92949062	2.61996091	0.00000408
H	3.45961911	0.57248460	-0.00001393
H	2.77939349	2.82451124	-0.00001309
H	1.26567030	3.11969007	0.87768262
H	1.26566698	3.11967503	-0.87770773
H	-3.80715943	-1.77726190	-0.00000794

E(PBE0) = -611.117848 Hartree
Dipole Moment = 7.242106 Debye

3.2 Cartesian coordinates optimized of 6,7-dihydroxy-4-methyl-2*H*-chromen-2-one (**4b**) at PBE0/aug-cc-pvTZ level theory using the SMD model solvation.

Charge = 0 Multiplicity = 1

O	-0.40616153	-1.49026392	-0.52006853
C	-1.13566310	-2.64057303	-0.40719225
C	-2.51579959	-2.51397427	-0.05842472
C	-3.10461014	-1.31039627	0.15782331
C	-2.80143734	1.17752089	0.22922296
C	1.98184173	2.27040801	0.09090200
C	-0.62849668	2.09623863	-0.25396010
C	-0.12064095	0.82904290	-0.45414164
C	-0.96148126	-0.26173051	-0.31044980
C	-2.30694463	-0.12090789	0.03048869
O	-2.38134217	3.56102538	0.26584110
O	0.18856984	3.16448559	-0.39322052
O	-0.53668709	-3.68474125	-0.61638163
C	-4.54386479	-1.21413823	0.51940788
H	-3.07483762	-3.43612953	0.02566698
H	-3.84071839	1.32987811	0.49522904

H	-3.31626982	3.58941551	0.49725555
H	-0.31285153	3.97214539	-0.22198217
H	-4.99864507	-2.20156150	0.57317235
H	-5.08554637	-0.61440939	-0.21661084
H	-4.66479261	-0.71563325	1.48466397
H	0.91955230	0.69190670	-0.71952530

E(PBE0) = -686.301412 Hartree
Dipole Moment = 11.940913 Debye

3.3 Cartesian coordinates optimized of 7-hydroxy-4-methyl-8-(piperidin-1-ylmethyl)-2*H*-chromen-2-one (**5a**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

C	-0.91869082	1.84654291	-1.3260873
C	-1.41288353	0.53773493	-1.2658547
C	-0.73810358	-0.45560557	-0.5442296
C	0.43290777	-0.08204014	0.1039812
C	0.95163883	1.21784955	0.0634680
C	0.24337877	2.17572841	-0.6741932
O	1.08331603	-1.06562018	0.7892996
C	2.24428382	-0.83905451	1.4743320
C	2.77790237	0.48438798	1.4476411
C	2.17139818	1.49234566	0.7704131
O	2.72603540	-1.80169211	2.0517762
C	2.75471230	2.86132706	0.7568669
O	-2.55309967	0.24905791	-1.9159738
C	-1.22781364	-1.87948440	-0.5381000
N	-2.68671206	-1.96266052	-0.6292880
C	-3.11197565	-3.28718770	-1.0821995
C	-4.61801998	-3.35395902	-1.2458598
C	-5.32763127	-2.97427821	0.0445026
C	-4.82683726	-1.62639023	0.5388236
C	-3.31483046	-1.62006197	0.6498849
H	-1.47092354	2.58537338	-1.8932338
H	0.61839744	3.18954927	-0.7277088
H	3.69711531	0.64334703	1.9950224
H	3.67293169	2.89799929	1.3405195
H	2.04666810	3.58566862	1.1670676
H	2.97424210	3.17646879	-0.2662487
H	-2.82580523	-0.68118319	-1.5766537
H	-0.86243620	-2.41021459	0.3480565
H	-0.81334419	-2.39562823	-1.4101764
H	-2.77991137	-4.04924950	-0.3591257
H	-2.61369020	-3.49728123	-2.0307786
H	-4.89386933	-4.36305646	-1.5610300
H	-4.91942988	-2.67130056	-2.0472929
H	-5.12486595	-3.73583431	0.8060431
H	-6.40976014	-2.95201485	-0.1047402
H	-5.25342455	-1.38414003	1.5150233
H	-5.13849721	-0.83808948	-0.1545000

H	-2.99041607	-2.33939347	1.4183310
H	-2.95698628	-0.63434027	0.9556422

E(PBE0) = -900.896891 Hartree
Dipole Moment = 5.163712 Debye

3.4 Cartesian coordinates optimized of 7-hydroxy-4-methyl-8-(pyrrolidin-1-ylmethyl)-2H-chromen-2-one (**5b**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

C	-0.80423409	1.91601282	-1.37849935
C	-1.31289818	0.61197303	-1.34769380
C	-0.68033692	-0.39252819	-0.60482368
C	0.46830329	-0.03598861	0.09164602
C	1.00134592	1.25852126	0.08119653
C	0.33316827	2.22897837	-0.67703341
O	1.08066506	-1.03116200	0.79482813
C	2.21598172	-0.82169350	1.52676727
C	2.76362072	0.49636164	1.53118712
C	2.19479624	1.51532436	0.83812455
O	2.66488633	-1.79344535	2.11515553
C	2.79210821	2.87814025	0.85759858
O	-2.42623350	0.33979279	-2.05104656
C	-1.18792371	-1.80965701	-0.62058682
N	-2.63846839	-1.87587906	-0.76855340
C	-3.11451607	-3.21826709	-1.10584411
C	-4.61722633	-3.15524251	-0.85276921
C	-4.79096472	-2.01410496	0.17179082
C	-3.37169742	-1.53685826	0.45463295
H	-1.32511445	2.66388215	-1.96315254
H	0.72001734	3.23930306	-0.70735611
H	3.66204608	0.64184064	2.11557598
H	3.68681861	2.90111378	1.47733102
H	2.07575560	3.60699942	1.24470001
H	3.05522910	3.19799374	-0.15372291
H	-2.72212453	-0.58684023	-1.73891501
H	-0.86936142	-2.34388035	0.28197977
H	-0.74395473	-2.33278280	-1.47404434
H	-2.63990668	-3.95457523	-0.44116072
H	-2.85068150	-3.47109053	-2.13350884
H	-4.99160325	-4.10909991	-0.48216819
H	-5.15291836	-2.92798549	-1.77495087
H	-5.28437704	-2.34254507	1.08615447
H	-5.38841070	-1.20615003	-0.25202279
H	-2.93823832	-2.08087531	1.30624569
H	-3.30377214	-0.46839577	0.66500825

E(PBE0) = -861.612039 Hartree
Dipole Moment = 5.104207 Debye

3.5 Cartesian coordinates optimized of 8-((diethylamino)methyl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (**5c**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

C	-0.94602478	1.74718755	-1.44610287
C	-1.39896328	0.42472321	-1.37732574
C	-0.72910351	-0.52922408	-0.60048600
C	0.39410683	-0.10502161	0.09819601
C	0.87202724	1.21059618	0.05012142
C	0.17214926	2.12731090	-0.74571639
O	1.04008261	-1.04979141	0.83973781
C	2.15819269	-0.76868528	1.57371713
C	2.65087451	0.57045143	1.53862363
C	2.04634177	1.54182572	0.80841652
O	2.64119706	-1.70030216	2.19910306
C	2.58611150	2.92845985	0.78892483
O	-2.49284070	0.07269141	-2.07390828
C	-1.18821038	-1.96123737	-0.60391297
N	-2.65385378	-2.06090556	-0.64007633
C	-3.04435333	-3.39066938	-1.11870711
C	-4.53223453	-3.56799140	-1.32455514
C	-4.46699897	-0.84274048	0.59960336
C	-3.23759138	-1.72679890	0.67388078
H	-1.49193428	2.45530308	-2.05682855
H	0.51755920	3.15137376	-0.80502265
H	3.53684241	0.77241387	2.12522616
H	3.47251323	3.00902233	1.41567333
H	1.83589057	3.63875976	1.14517551
H	2.84627851	3.22626578	-0.22987909
H	-2.78380694	-0.82375035	-1.66735868
H	-0.77534735	-2.50787773	0.24953080
H	-0.80430601	-2.44485549	-1.50760845
H	-2.67513069	-4.15477219	-0.41801533
H	-2.52973350	-3.55263860	-2.06841913
H	-4.71467342	-4.55303442	-1.75889458
H	-4.93148829	-2.81704885	-2.00970916
H	-4.84315917	-0.65630590	1.60828384
H	-4.22657953	0.12167018	0.14800987
H	-3.46277547	-2.65511965	1.21261176
H	-2.47452324	-1.20865668	1.25842091
H	-5.27063330	-1.29797932	0.02069764
H	-5.08914911	-3.51425668	-0.38816945

E(PBE0) = -862.806697 Hartree
Dipole Moment = 4.975896 Debye

3.6 Cartesian coordinates optimized of 6,7-dihydroxy-4-methyl-8-(piperidin-1-ylmethyl)-2*H*-chromen-2-one (**5d**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

O	-0.35143423	-1.46122861	-0.44623993
C	-1.06101598	-2.61665333	-0.33612169
C	-2.44762580	-2.50904754	-0.01163397
C	-3.05233523	-1.30876734	0.17232944
C	-2.80029297	1.17585592	0.20195428
C	-1.99702790	2.27618700	0.05443734
C	-0.63289958	2.12336046	-0.24845098
C	-0.06128051	0.86618624	-0.40345205
C	-0.91075348	-0.23228542	-0.26315678
C	-2.26848218	-0.11063093	0.04080124
O	-2.42481545	3.56528202	0.17765714
O	0.13471621	3.22476798	-0.40499673
C	1.39636830	0.73040028	-0.74843057
N	2.28208347	0.87105710	0.41021385
O	-0.44328960	-3.65501372	-0.52693830
C	3.66182317	1.13549490	-0.00046326
C	4.38666656	-0.05938091	-0.60757456
C	4.35577745	-1.23813371	0.35542166
C	2.92227108	-1.53814921	0.77210916
C	2.24407064	-0.28300384	1.31037099
C	-4.50018212	-1.22298963	0.50361600
H	-2.99595708	-3.43710023	0.07694108
H	-3.84887857	1.31086761	0.43778682
H	-3.36982793	3.58420437	0.36532613
H	-0.42046425	4.00674080	-0.28676159
H	1.65360900	1.53123668	-1.44357758
H	1.54333119	-0.21045490	-1.28866193
H	4.21034571	1.45667761	0.89233835
H	3.65771666	1.97885450	-0.69497490
H	5.41617413	0.22768325	-0.83985352
H	3.91537220	-0.34141794	-1.55500696
H	4.94453593	-0.98631434	1.24604227
H	4.82132690	-2.11983632	-0.09292299
H	2.89508071	-2.31249358	1.54412314
H	2.36528041	-1.93008904	-0.08420466
H	2.74886073	0.01459195	2.23618892
H	1.20443852	-0.48816549	1.57392377
H	-4.94260610	-2.21518099	0.57249290
H	-5.03553870	-0.64992135	-0.25780409
H	-4.64781230	-0.70356828	1.45384654

E(PBE0) = -976.069168 Hartree
Dipole Moment = 10.283564 Debye

3.7 Cartesian coordinates optimized of 6,7-dihydroxy-4-methyl-8-(pyrrolidin-1-ylmethyl)-2*H*-chromen-2-one (**5e**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

O	-0.30555982	-1.38545660	-0.50357691
C	-0.96101599	-2.57488624	-0.42068608

C	-2.35115299	-2.53868420	-0.09569081
C	-3.00977061	-1.37244364	0.11961934
C	-2.86991120	1.11987500	0.21750125
C	-2.11752701	2.25889457	0.09778796
C	-0.74906965	2.17622788	-0.21279789
C	-0.12206370	0.95084159	-0.40259152
C	-0.92030292	-0.18876770	-0.28832120
C	-2.28108632	-0.13671413	0.02082023
O	-2.60094281	3.52419370	0.25471979
O	-0.03337963	3.31476821	-0.34404820
C	1.33392046	0.88688770	-0.76391909
N	2.23744849	1.04525656	0.38725772
O	-0.29552388	-3.57862428	-0.63423610
C	3.62839184	1.08090019	-0.08170361
C	4.05124710	-0.37171600	-0.34893125
C	3.12370725	-1.19879606,	0.55997934
C	2.24133558	-0.14459215	1.24720402
C	-4.45999535	-1.36183907	0.45148624
H	-2.85690779	-3.49262849	-0.03234850
H	-3.92257891	1.20100027	0.45929120
H	-3.54632137	3.49842954	0.43930741
H	-0.62273810	4.06753038	-0.20286168
H	1.54904242	1.70651660	-1.45053273
H	1.51935435	-0.04266860	-1.31290483
H	4.24300246	1.50053816	0.71874783
H	3.72392799	1.73172328	-0.95099644
H	5.10673885	-0.52274049	-0.11987569
H	3.90599123	-0.63485520	-1.39778846
H	3.67525839	-1.78524983	1.29573302
H	2.52150395	-1.89563809	-0.02460207
H	2.68718039	0.13867508	2.20391887
H	1.22688227	-0.48518029	1.45020407
H	-4.85747304	-2.37455881	0.48789776
H	-5.02031893	-0.78893697	-0.29180443
H	-4.63065295	-0.88066685	1.41784844

E(PBE0) = -936.786401 Hartree
Dipole Moment = 10.259344 Debye

3.8 Cartesian coordinates optimized of 8-((diethylamino)methyl)-6,7-dihydroxy-4-methyl-2*H*-chromen-2-one (**5f**) at PBE0/aug-cc-pvTZ level using the SMD model solvation.

Charge = 0 Multiplicity = 1

O	-0.36923776	-1.53541740	-0.44327886
C	-1.07863885	-2.69062082	-0.33121130
C	-2.46887965	-2.58160874	-0.02312955
C	-3.07616120	-1.38051094	0.14776112
C	-2.82605257	1.10586202	0.16011313
C	-2.02095977	2.20458126	0.00822226
C	-0.65482856	2.04929870	-0.28334869
C	-0.08231734	0.79115393	-0.42188581

C	-0.93235296	-0.30570738	-0.27783629
C	-2.29262637	-0.18223167	0.01457030
O	-2.45049122	3.49442204	0.11473284
O	0.11553502	3.14829958	-0.44650919
C	1.38054074	0.65457649	-0.74935295
N	2.24999746	0.79215373	0.41336792
O	-0.45675908	-3.72948888	-0.50511666
C	3.59942678	1.21664016	0.05477208
C	4.33383496	0.46185290	-1.04886623
C	2.74485917	-1.66756819	0.96006586
C	2.17341440	-0.31133710	1.36158628
C	-4.52682794	-1.29492136	0.46604178
H	-3.01771567	-3.50941020	0.06579857
H	-3.87658624	1.24403904	0.38547116
H	-3.39798595	3.51094328	0.28811395
H	-0.43743609	3.93395940	-0.34300592
H	1.64205069	1.45628449	-1.44247258
H	1.53587989	-0.28544689	-1.29089124
H	4.19523677	1.19130062	0.97111084
H	3.54260482	2.26817334	-0.24842726
H	5.30916701	0.92947221	-1.20761886
H	3.79404001	0.51031215	-1.99704209
H	2.45298197	-2.41317967	1.70452567
H	2.36983009	-2.00574091	-0.00759253
H	2.67111136	0.01663520	2.27917735
H	1.11874911	-0.43728110	1.62121309
H	-4.96983522	-2.28710571	0.52999657
H	-5.05554694	-0.72075865	-0.29909311
H	-4.68250943	-0.77649556	1.41555148
H	4.50264491	-0.58735760	-0.80818336
H	3.83488727	-1.65401546	0.92089910

E(PBE0) = -937.981135 Hartree
Dipole Moment = 10.565352 Debye

3.9 Theoretical calculations performed at several levels of theory, the final decision was made by accuracy with experimental data and other results already published.

Table 1. Topological indexes of electronic density in critical points for selected bonds of compounds **5a-5f** calculated at B3LYP/aug-cc-pVTZ level of theory.

	Entry	$\nabla^2\rho(ea_0^{-5})$	ϵ	**V(a.u.)**	H_b**(a.u.)**	H_b**(kcal/mol)**
N13-H7	**5a**	0.1235	0.0244	-0.0417	-0.0054	-13.0836
	5b	0.1239	0.0245	-0.0419	-0.0055	-13.1463
	5c	0.1301	0.0181	-0.0453	-0.0064	-14.2131
O6-H7	**5d**	0.0855	0.6394	-0.0182	0.0016	-5.7103
	5e	0.0855	0.6345	-0.0183	0.0015	-5.7417

5f	0.0863	0.5779	-0.0186	0.0015	-5.8358

Table 2. Topological indexes of electronic density in critical points for selected bonds of compounds **5a-5f** calculated at PBE0/aug-cc-pVTZ level of theory.

	Entry	$\nabla^2\rho(ea_0^{-5})$	ϵ	**V(a.u.)**	H_b**(kcal/mol)**
N13-H7	**5a**	0.1554	0.01540	-0.0687	-21.55
	5b	0.1557	0.0176	-0.0655	-20.55
	5c	0.1527	0.0182	-0.0658	-20.65
O6-H7	**5d**	0.0873	1.0574	-0.0190	-5.96
	5e	0.0876	1.0109	-0.0191	-5.99
	5f	0.0869	1.2087	-0.0188	-5.90

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at B3LYP/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-6.326	-1.892	4.434	-611.8156
5a	-6.134	-1.823	4.310	-901.9404
5b	-6.146	-1.828	4.319	-862.6064
5c	-6.152	-1.830	4.321	-863.8132
4b	-6.078	-1.901	4.178[a]	-687.0728
5d	-5.729	-1.860	3.869	-977.1871
5e	-6.045	-1.866	4.178	-937.8548
5f	-5.652	-1.856	3.797	-939.0624

[a] $\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at ωB97XD/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-8.176	0.005	8.181	-611.6022
5a	-7.996	0.053	8.049	-901.6474

5b	-8.012	0.049	8.061	-862.3232
5c	-8.028	0.043	8.071	-863.5295
4b	-7.916	-0.012	7.904[a]	-686.8372
5d	-7.828	0.015	7.843	-976.8746
5e	-7.894	0.008	7.902	-937.5519
5f	-7.902	0.017	7.799	-938.7581

[a]$\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at M06-2X/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-7.621	-0.933	6.688	-611.5788
4b	-7.376	-0.954	6.421[a]	-686.8150

[a]$\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at M06/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-6.527	-1.602	4.925	-611.4037
4b	-6.265	-1.613	4.652 [a]	-686.6300

[a]$\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at PBE0/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-6.523	-1.700	4.823	-611.1178
5a	-6.317	-1.633	4.683	-900.8969
5b	-6.330	-1.636	4.693	-861.6120
5c	-6.333	-1.640	4.694	-862.8067
4b	-6.250	-1.705	4.545[a]	-686.3014
5d	-5.979	-1.671	4.308	-976.0692

5e	-6.223	-1.678	4.545	-936.7864
5f	-5.920	-1.669	4.251	-937.9811

[a] $\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 2. Molecular orbital energies (E_{HOMO} and E_{LUMO}, eV), gap energies (ΔE_g, eV), and total energy (E_{Tot}, hartree) of **4a**, **4b**, and **5a-5f** compounds calculated at HSE/aug-cc-pVTZ level of theory.

Compound	E_{HOMO}	E_{LUMO}	ΔE_g	E_{Tot}
4a	-6.144	-2.068	4.076	-611.1716
4b	-5.872	-2.073	3.799[a]	-686.3618

[a] $\Delta E_{g\text{-reported}}$= 4.154 eV in ref. 24.

Table 3. Calculated maximum absorption wavelengths λ_{max} (nm), oscillator strengths (*f*), transition energy ($E_{g\text{-theo}}$, eV), % of contribution, and assignment of **4a**, **4b**, and **5a-5f** compounds calculated at B3LYP/aug-cc-pVTZ level of theory.

Compound	λ_{max}	*f*	$E_{g\text{-theo}}$	%Major Contribution	Assignment
4a	327.41 [a]	0.7058	3.7869 [b]	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
5a	330.99	0.5949	3.7459	HOMO->LUMO (98%)	$\pi \rightarrow \pi^*$
5b	336.95	0.6777	3.6796	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
5c	336.73	0.6797	3.6820	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
4b	348.25	0.5558	3.5602	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
5d	378.83	0.0046	3.2728	HOMO->LUMO (100%)	$n \rightarrow \pi^*$
5e	348.24	0.5373	3.5603	HOMO->LUMO (99%)	$n \rightarrow \pi^*$
5f	388.32	0.0042	3.1928	HOMO->LUMO (100%)	$n \rightarrow \pi^*$

[a] λ_{max}=325 nm in toluene solvent in ref. 23 and [b] $E_{g\text{-theo}}$=319nm–323nm in different organic solvents in ref. 32.

Table 3_new. Calculated maximum absorption wavelengths λ_{max} (nm), oscillator strengths (*f*), transition energy ($E_{g\text{-theo}}$, eV), % of contribution, and assignment of **4a**, **4b**, and **5a-5f** compounds calculated at PBE0/aug-cc-pVTZ level of theory.

Compound	λ_{max}	*f*	$E_{g\text{-theo}}$	%Major Contribution	Assignment

4a	300.73 [a]	0.4392	4.1227 [b]	HOMO->LUMO (100%)	$\pi \rightarrow \pi^*$
5a	310.79	0.3997	3.9893	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
5b	310.06	0.4010	3.9987	HOMO->LUMO (100%)	$\pi \rightarrow \pi^*$
5c	310.06	0.4017	3.9987	HOMO->LUMO (100%)	$\pi \rightarrow \pi^*$
4b	322.38	0.3282	3.8458	HOMO->LUMO (99%)	$\pi \rightarrow \pi^*$
5d	348.71	0.0038	3.5556	HOMO->LUMO (98%)	$n \rightarrow \pi^*$
5e	322.91	0.2948	3.8392	HOMO->LUMO (100%)	$n \rightarrow \pi^*$
5f	355.49	0.0031	3.4877	HOMO->LUMO (98%)	$n \rightarrow \pi^*$

[a] λ_{max}=325 nm in toluene solvent in ref. 23 and [b] $E_{g\text{-}theo}$=319nm–323nm in different organic solvents in ref. 32.

4. Test to verify the authenticity of Human Cell Line

SALUD
SECRETARÍA DE SALUD

Instituto Nacional de Medicina Genómica
MÉXICO

Laboratorio de
Diagnóstico Genómico

Test to verify the authenticity Human Cell Line.

Result issued on: November 23, 2020 14:19 hrs

Methodology: DNA extraction was performed from the provided sample using the appropriate methodology according to the type of sample delivered (Maxwell 16 from Promega. QIAIAamp DNA Blood Mini Kit from QIAGEN Prep-n-go buffer from AB or FTA Purification Reagent from GE). Multiplex-type polymerase chain reaction and capillary electrophoresis on AB's 3500 Genetic Analyzer, fragment analysis on Gene Mapper ID v5.

Results:

Marcador	LCH1120-220-33
D3S1358	15 18
vWA	16 18
D16S539	9 9
CSF1PO	9 10
TPOX	8 12
D8S1179	12 12
D21S11	27 28
D18S51	13 16
Penta E	7 17
D2S441	10 11
D19S433	13 14
TH01	7 7
FGA	18 21
D22S1045	16 17
D5S818	11 12
D13S317	12 13.3
D7S820	8 12
D6S1043	18 18
D10S1248	13 15
D1S1656	12 15
D12S391	20 25
D2S1338	17 17
Penta D	8 15
Amelogenina	X X

From the short tandem repeat (STR) DNA profiles, identity of the cell line was corroborated at CLASTR, which is an STR profile search tool available through Cellosaurus (https://web.expasy.org/cellosaurus-str-search/). According with this database, cells were authenticated as a **derivative of HeLa cell line**.