Electronic Supporting Information

Identification of karanjin isolated from Indian beech tree as potent CYP1 enzyme inhibitor with cellular efficacy via screening of a natural products repository

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S1. EXPERIMENTAL PROCEDURES

In-vitro CYP450 enzyme inhibition

All CYP enzymes (Saccharosomes^{TM}; human CYP enzymes bound to yeast microsomal membranes) used in this study were manufactured by CYP Design Ltd (Leicester, UK). This method was used to measure the percentage inhibition of a CYP450 by a compound or to determine the IC_{50} values (the concentration at which 50% of the enzyme activity is inhibited) of a compound. Both percentage inhibition and IC_{50} values effectively reflect the inhibitory potential of a compound and hint at the possible effectiveness of a compound in a biological process. Percentage inhibition is determined at a particular concentration of the compound which is usually 10 μM. An assay which determines IC_{50} values includes the yeast microsomes that bear the cytochrome P450 enzymes (i.e. Sacchrosomes^{TM}), a chosen chemical compound in six serial dilutions in DMSO (with DMSO concentration never exceeding 0.5%), 96-well flat-bottomed microtitre plate, substrates such as ER (7-ethoxyresorufin) or CEC (3-cyano-7-ethoxycoumarin) or EOMCC (7-ethoxy-methyloxy-3-cyanocoumarin) or DBF (dibenzylfluorescein), depending on the CYP450 used in the assay. The substrates form fluorescent compounds upon CYP metabolism. A fluorescent plate reader is used to monitor fluorescence emitted which ultimately determines IC_{50} values via measurement of fluorescence units at each endpoint (i.e. at each concentration of compound used). The basal fluorescence of the test compounds is subtracted from the measured fluorescence at each concentration point. before calculation of % inhibition and IC_{50} value.

A typical CYP450 end point assay, for inhibition of CYP1B1

Regenerating system consists of: 5 μL Solution A (183 mg of $NADP^+$ + 183 mg of glucose-6-phosphate + 654 μL of 1.0 M magnesium chloride solution + 9.15 mL of sterile

ultra-pure water) + 1 µL Solution B (250 Units of glucose-6-phosphate dehydrogenase + 6.25 mL of 5 mM sodium citrate; mixed in a tube and made up to 10 ml with sterile ultra-pure water) + 39 µL 0.2 M phosphate buffer (KPi; 0.6 mL of 1.0M K2HPO4 + 9.4 mL of 1.0 M KH_2PO_4 mixed and made up to 50 mL with sterile ultra-pure water) + 5 µL potential inhibitory compound. Enzyme system consists of: 0.5 µL CYP1B1 (0.5 pmoles; CYP Design Ltd) + 1.7 µL control protein (denatured proteins from yeast cells that do not contain recombinant CYP450 proteins) + 5 µL 0.1 mM 7-ER (7-ethoxyresorufin substrate) + 42.8 µL 0.1M Kpi (0.3 mL of 1.0 M K_2HPO_4 + 4.7 mL of 1.0 M KH_2PO_4 were mixed and made up to 50 mL with sterile ultra-pure water. The assay is performed using (a) sensitivity (Gain): 65/70/75 of the Biotek Synergy plate reader (this would differ from one instrument to the other) and (b) Filter: 530/590 nm that monitors fluorescence excitation/ emission of resorufin, the metabolite of 7-ethoxyresorufin substrate (ER); the excitation/ emission differs with the substrate that is used. Similar assays were performed with Sacchrosomes™ bearing the other human CYPs using appropriate fluorescent substrates, as detailed above.

Procedure for IC_{50} determination using Sacchrosomes™

The plate reader (BioTek) was warmed at 37 °C. Compounds were serially diluted to six different concentrations with 10% DMSO in a Sero-Wel white microplate. Serial dilutions were made with a dilution factor of 1:20. 45 µL of regenerating system was prepared and pre-warmed at 37 °C, as detailed in Table 1.

Table S1A. The constitution of the regenerating system used per reaction in each single well for different CYPs was as follows.

Enzyme	Solution A	Solution B	Inhibitor	KPi buffers	water
CYP1A1	5 µL	1 µL	5 µL	39 µL 0.2 M	-
CYP1B1	5 µL	1 µL	5 µL	39 µL 0.2 M	-
CYP1A2	5 µL	1 µL	5 µL	20 µL 0.5 M	19 µL
CYP2D6	5 µL	1 µL	5 µL	25 µL 0.2 M	14 µL

CYP3A4	5 µL	1 µL	5 µL	25 µL 0.2 M	14 µL

Meanwhile, 50 µL of enzyme substrate mix reaction was prepared and incubated at 37°C for 10 min (Table 2).

Table S1B. The constitution of enzyme-substrate mixtures was as follows.

Enzyme	P450 conc. in Sacchrosomes™	Control Microsome	Substrate	KPi buffers	water
CYP1A1	0.5 µL (0.5 pmole)	2 µL	5 µL 0.1 mM E.R.	42.5 µL 0.1 M	-
CYP1B1	0.5 µL (0.5 pmole)	1.7 µL	5 µL 0.1 mM E.R.	42.8 µL 0.1 M	-
CYP1A2	1 µL (1 pmole)	1.6 µL	5 µL 320 µM CEC	42.4 µL 0.1 M	-
CYP2D6	2.5 µL (2.5 pmole)	0.4 µL	0.5 µL 2 mM EOMCC	25 µL 0.2 M	21.6 µL
CYP2C9	1 µL (1 pmole)	1.6 µL	5 µL 320 µM CEC	42.4 µL 0.1 M	-
CYP2C19	1 µL (1 pmole)	1.6 µL	5 µL 320 µM CEC	42.4 µL 0.1 M	-
CYP3A4	1.1 µL (1 pmole)	10.102 µL	0.1 µL 2 mM	25 µL 0.2 M	23.96 µL

In wells of a black 96-well flat-bottomed microplate, 45 µL of regenerating system, 5 µL serial dilutions of inhibitor were pipetted out from the dilution plate and then 50 µL of enzyme/substrate was added except in control well (positive control); for this well, instead of inhibitor 5 µL of 10% DMSO was added. In the background well (negative control), only 45 µL regenerating system and 5 µL 10% DMSO were added with no enzyme; the microplate was then vortexed for a few seconds. The microplate was incubated for 10 min. which was followed by addition of 75 µL of Tris-acetonitrile to all wells, using an eight-channel multi-pipette, to stop the reaction; after that 50 µL of enzyme/substrate reaction was added into the ‘ negative control’ well. The plate was left to shake for 10 sec and the fluorescence units for each endpoint were monitored at appropriate settings (for assay parameters and plate layout) selected on the KC4 software of the BioTek plate reader.

Calculation of IC_{50} values

To calculate IC_{50} values, a series of dose-response data, for example, drug concentrations (x_1, x_2, ...,x_n) at which specific growth inhibition occurs (y_1, y_2, ...,y_n) were generated. The values of y were in the range of 0-1. The simplest estimate of IC_{50} is to plot x-y and fit the data with a straight line (via linear regression). IC_{50} values are then estimated using the fitted line, i.e.

$Y = a * X + b,$

$IC_{50} = (0.5 - b)/a.$

Raw data was imported and computed in Microsoft Excel. The maximum change in relative fluorescence units (RFU) relative to positive control with 0.5% DMSO was calculated. The enzyme inhibition was plotted using sigmoidal curve (4 parameter variable slope equation) and half inhibitory concentration (IC_{50}) values were analysed statistically using Graph-Pad Prism Software (Version 6.0).

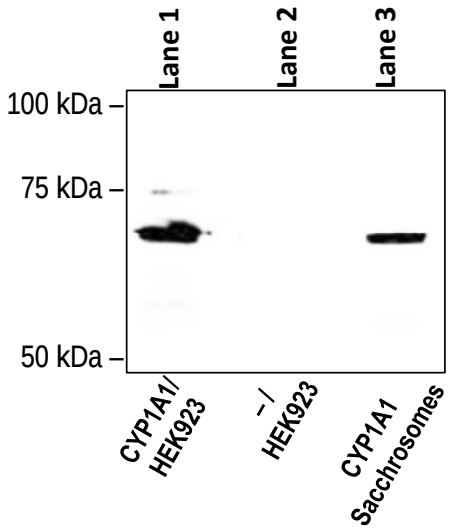
Transfection of mammalian expression plasmids that encode human *CYP1A1* & *CYP1B1* genes isolated from a human liver cDNA library in HEK293 cells grown in suspension cells

HEK293 'suspension' cells (1 x 10^6 per mL), obtained from CYP Design Ltd, were counted using improved Neubauer counting chamber and the cell viability (≥ 90% viability) was determined using trypan blue dye exclusion. Actively dividing suspension cells in log phase were seeded in appropriate volumes in Erlenmeyer flask (Corning #431143) and incubated at 37 °C, 8% CO_2 and shaken at 130 rpm on an orbital shaker (Panasonic). Before transfection, all mammalian expression plasmids containing human *CYP* genes (isolated from a human liver cDNA library) were propagated in *E. coli* DH5α, grown in LB medium in presence of ampicillin (50 µg/mL). The endotoxin-free plasmids

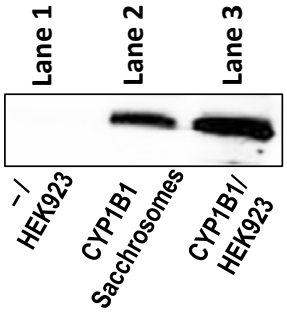
were prepared using Zymo PURE™ Plasmid Maxiprep Kit as per manufacturer's instructions (#D4202, Zymo Pure). The quantity and purity of plasmid DNA ($A_{260/280} \geq 1.9$) was determined by Bio Spectrophotometer (Eppendorf). The quality of plasmid DNA was determined using 1% agarose gel electrophoresis.

To initiate transfection, the respective plasmid DNA–cationic lipid complexes were prepared as per manufacturer's instructions (Invitrogen #16447-100) in OptiPRO SFM reduced serum medium (Invitrogen #12309-09). Further, the aseptic preparation of DNA-lipid complexes was added slowly to the respective flasks containing HEK293 suspension cells. The negative control was prepared by adding OptiPRO SFM reduced serum media without plasmid DNA. The suspension cells were incubated at 37 °C 24 to 48 h post transfection, the cells were counted and the cell viability was determined. The transfected cells in sufficient volumes were spun at 200 x g for 5 minutes. The supernatant was discarded and the cells were washed once with pre-warmed phosphate buffered saline. The cells were once again spun at 200 x g for 5 min at room temperature and the supernatant was discarded. The cells were gently re-suspended in pre-warmed growth media to obtain cell density ~4 x 10^6 transfected HEK293 cells per mL.

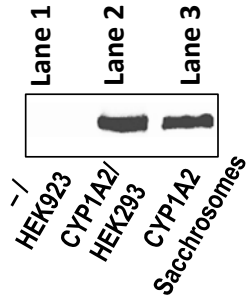
Western blot showing levels of expression of CYPs 1A1, 1B1, 1A2, 2D6, 2C9, 2C19, 3A4 measured in recombinant HEK293 cells



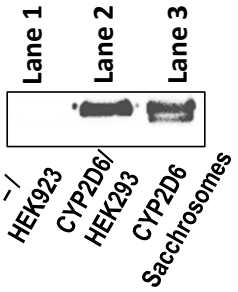
Western blot shows expression of CYP1A1 from the pcDAN3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP1A1 rabbit polyclonal antibody (Abcam; ab124295). Lane 1, cells which express CYP1A1 from the pcDNA3.1 plasmid; lane 2, cells which contain the empty plasmid pcDNA3.1; lane 3, CYP1A1 Sacchrosomes produced from baker's yeast.



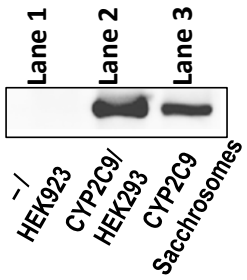
Western blot shows expression of CYP1B1 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP1B1 rabbit polyclonal antibody (Abcam; ab32649). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 0.5 pmole CYP1B1 Sacchrosomes produced from baker's yeast; lane 3, 1X 10^7 cells which express CYP1B1 from the pcDNA3.1 plasmid.



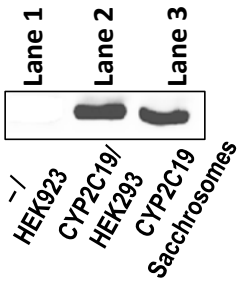
Western blot shows expression of CYP1A2 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP1A2 rabbit polyclonal antibody (Abcam; ab22717*). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 1X 10^7 cells which express CYP1A2 from the pcDNA3.1 plasmid; lane 3, 0.5 pmole CYP1A2 Sacchrosomes produced from baker's yeast.*



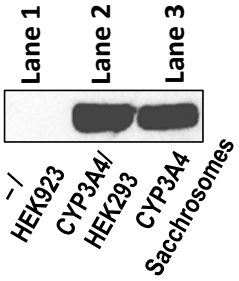
Western blot shows expression of CYP2D6 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP2D6 rabbit polyclonal antibody (Abcam; ab62204*). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 1X 10^7 cells which express CYP2D6 from the pcDNA3.1 plasmid; lane 3, 0.5 pmole CYP2D6 Sacchrosomes produced from baker's yeast.*



Western blot shows expression of CYP2C9 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP2C9 rabbit polyclonal antibody (Abcam; ab4236*). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 1X 10^7 cells which express CYP2C9 from the pcDNA3.1 plasmid; lane 3, 0.5 pmole CYP2C9 Sacchrosomes produced from baker's yeast.*

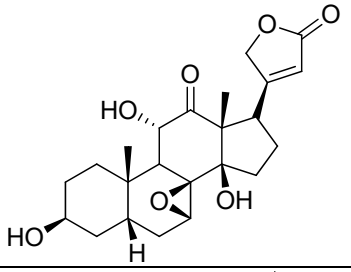
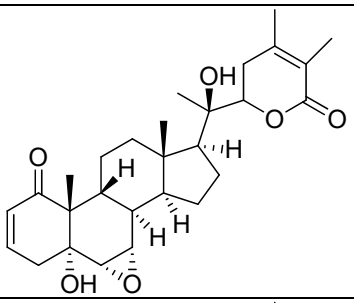
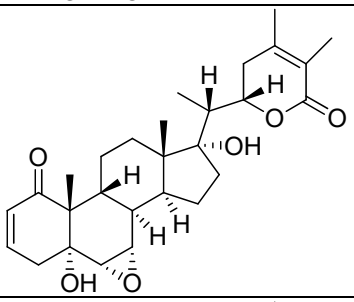
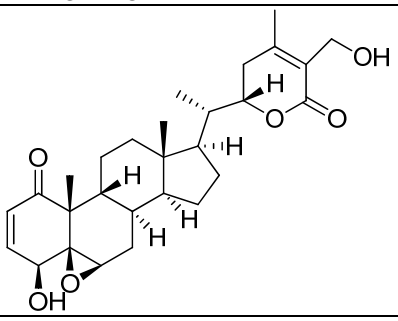
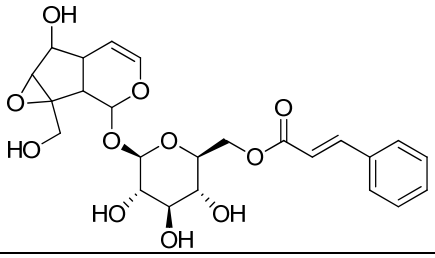


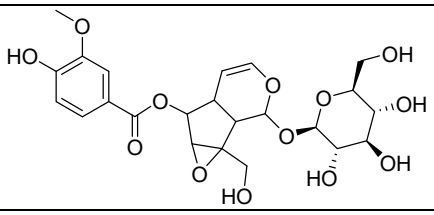
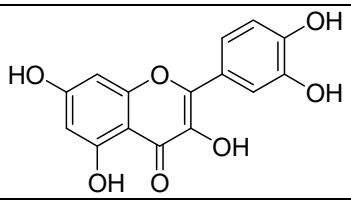
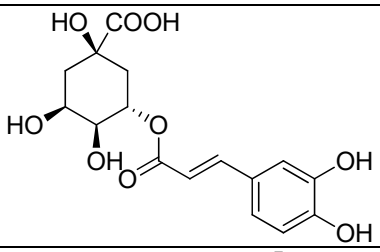
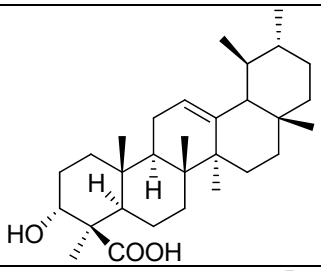
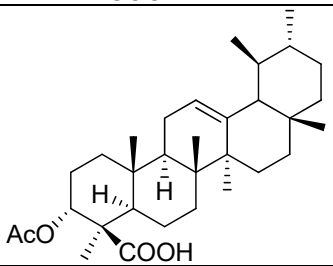
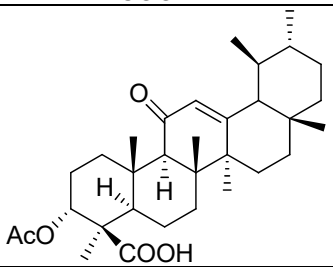
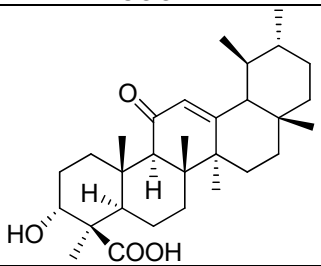
Western blot shows expression of CYP2C19 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP2C19 rabbit polyclonal antibody (Abcam; ab137015*). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 1X 10^7 cells which express CYP2C19 from the pcDNA3.1 plasmid; lane 3, 0.5 pmole CYP2C19 Sacchrosomes produced from baker's yeast.*

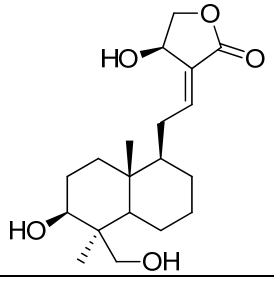
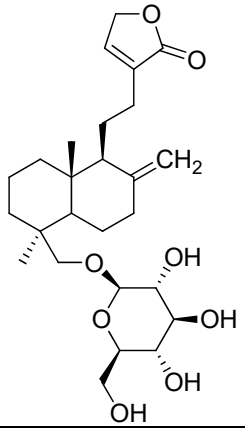
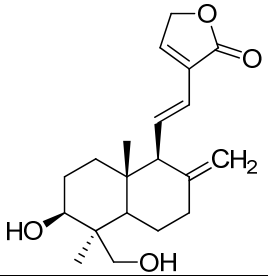
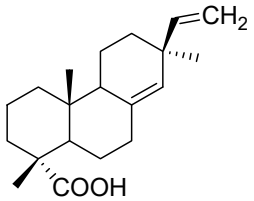
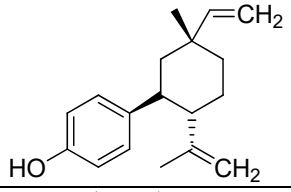
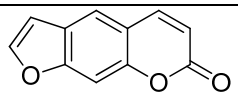
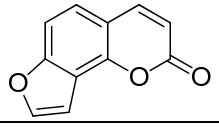


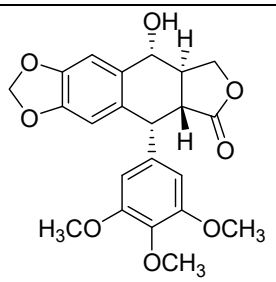
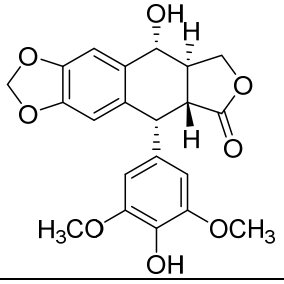
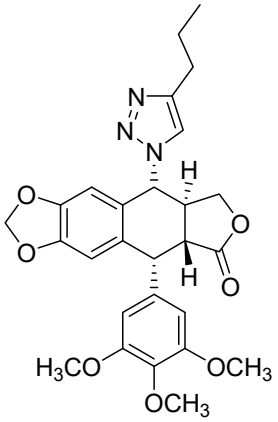
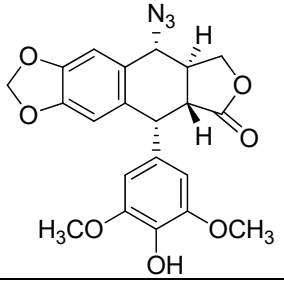
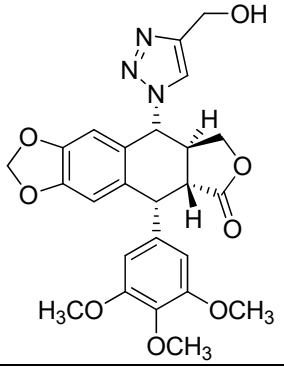
Western blot shows expression of CYP3A4 from the pcDNA3.1 plasmid when transfected in HEK293 cells. The Western blot was probed with a CYP3A4 rabbit polyclonal antibody (Abcam; ab3572*). Lane 1, 1X 10^7 cells which contain the empty plasmid pcDNA3.1; lane 2, 1X 10^7 cells which express CYP3A4 from the pcDNA3.1 plasmid; lane 3, 1.0 pmole CYP3A4 Sacchrosomes produced from baker's yeast.*

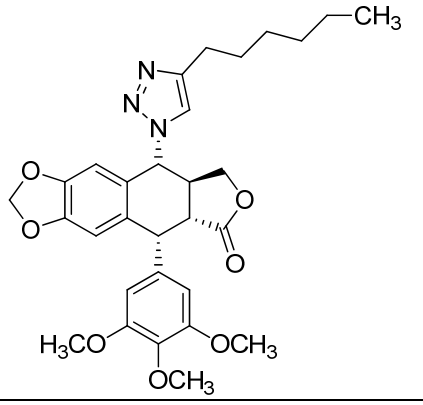
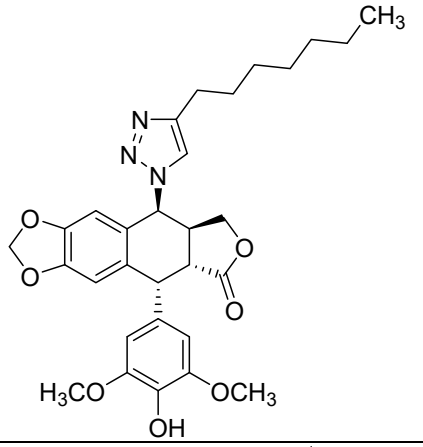
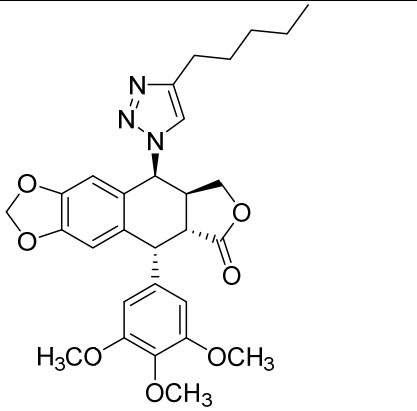
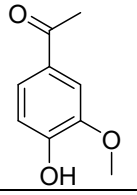
S2. Table S1. CYP1A1 screening results in Sacchrosomes™ [a]

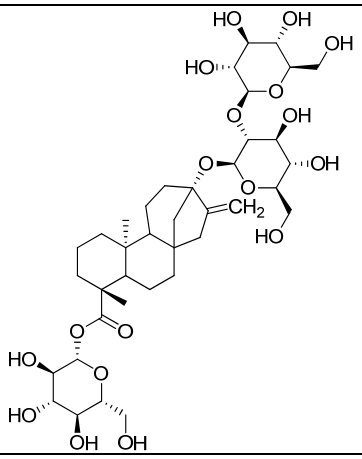
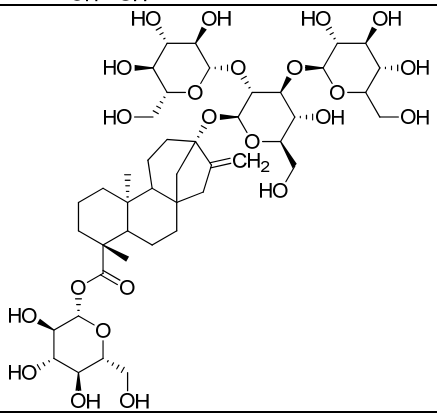
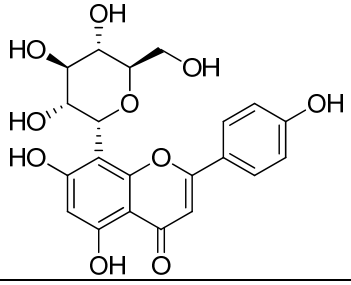
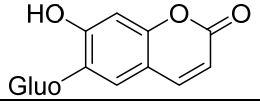
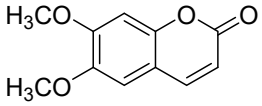
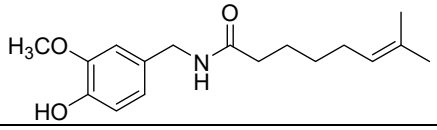
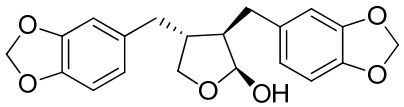
S. N.	Structure	Codes	Mol. Wt.	% CYP1A1 Inhibition at 10 µM
1		IN00001	418.48	23.1
2		IN00002	470.60	11
3		IN00003	470.60	17
4		IN00004	470.60	49.8
5		IN00005	492.47	14

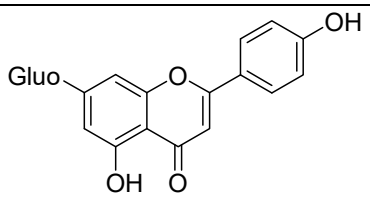
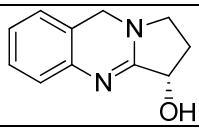
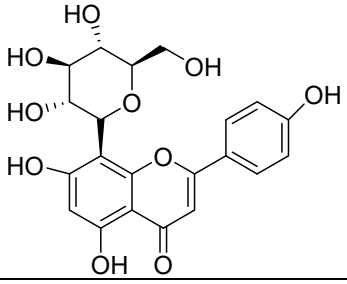
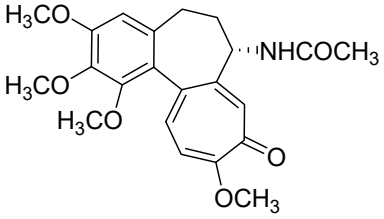
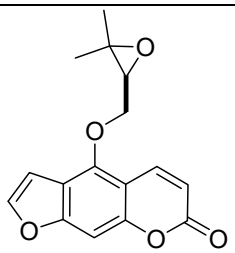
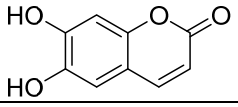
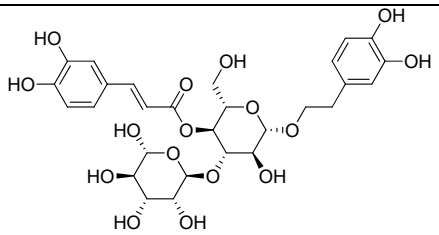
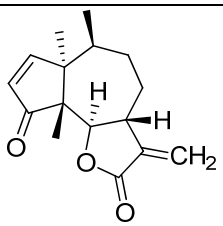
6		IN00006	512.46	17
7		IN00007	302.24	40
8		IN00008	354.31	No inhibition
9		IN00009	456.70	19
10		IN00010	498.74	6.6
11		IN00011	512.72	5
12		IN00012	470.68	7

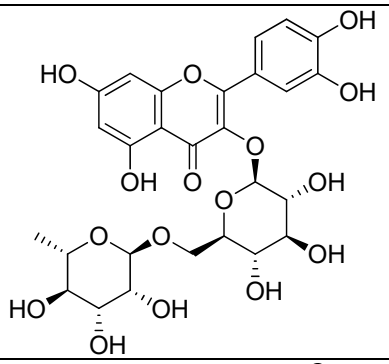
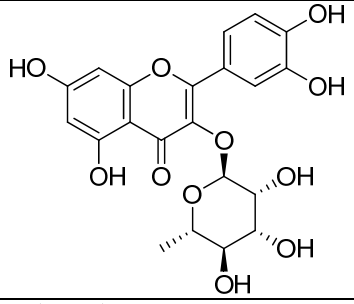
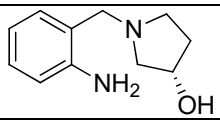
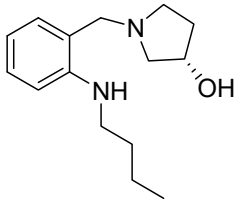
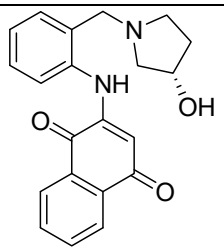
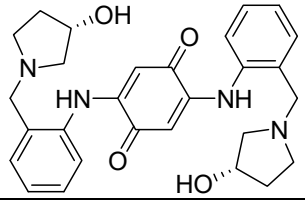
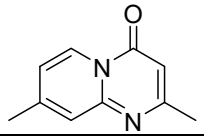
13		IN00013	338.44	12.5
14		IN00014	480.59	10.9
15		IN00015	332.43	7.7
16		IN00016	302.47	1
17		IN00018	256.38	7
18		IN00019	186.16	68.7
19		IN00020	186.16	80

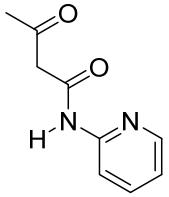
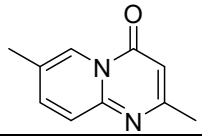
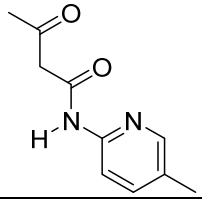
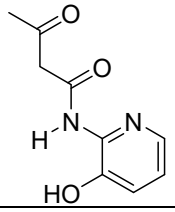
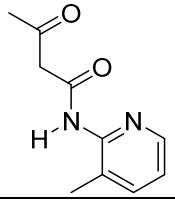
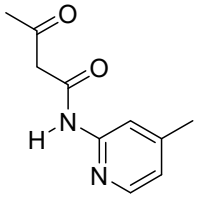
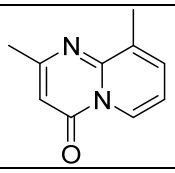
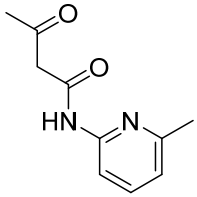
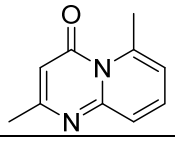
20	 Podophyllotoxin	IN00021	414.41	5.1
21		IN00022	400.38	No inhibition
22		IN00023	507.2	2
23		IN00024	426.40	5
24		IN00025	495.48	No inhibition

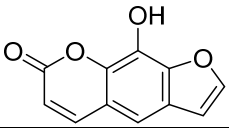
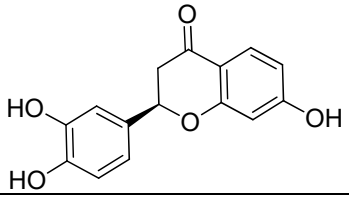
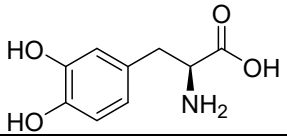
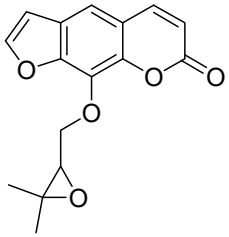
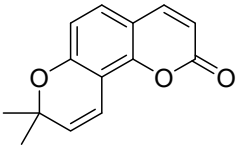
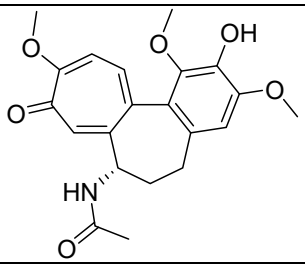
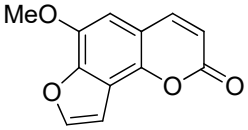
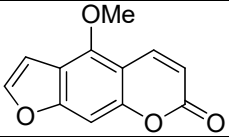
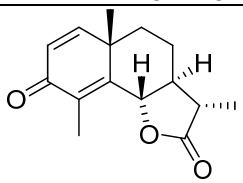
25		IN00026	549.61	8
26		IN00027	549.61	5
27		IN00038	535.59	6
28		IN00039	166.17	4

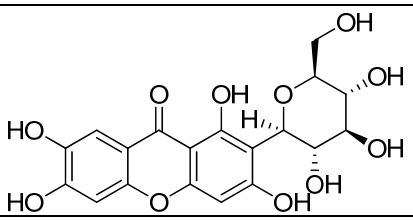
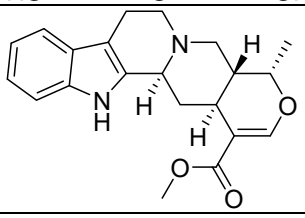
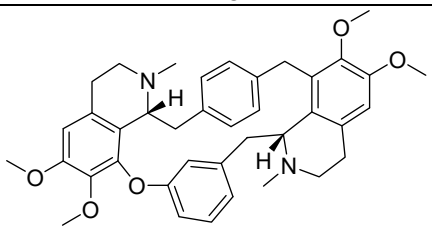
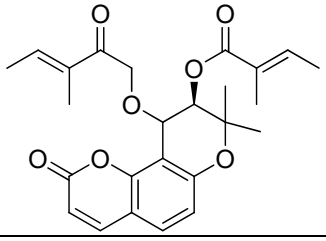
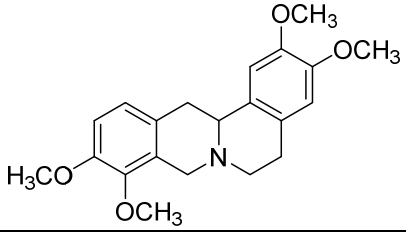
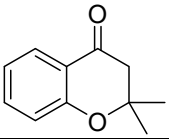
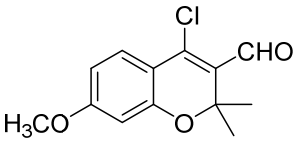
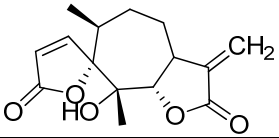
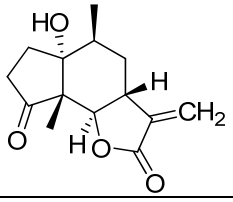
29		IN00040	804.87	33.9
30		IN00041	967.01	10.6
31		IN00042	432.38	3
32		IN00043	161.13	2
33[b]	 Scoparone	IN00044	206.19	2
34		IN00045	291.39	5
35		IN00046	356.37	14

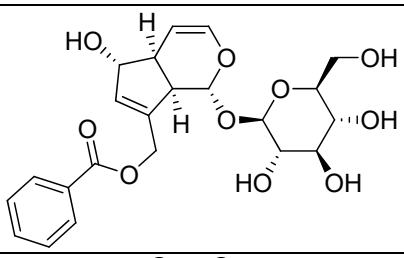
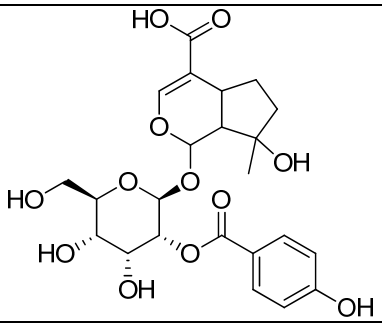
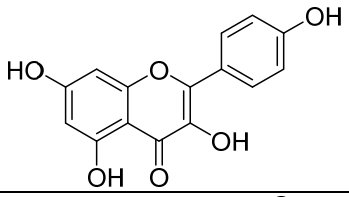
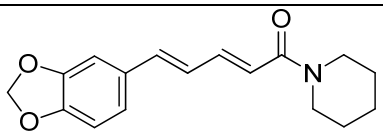
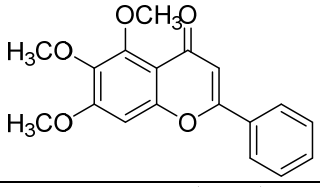
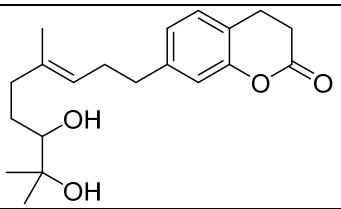
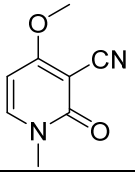
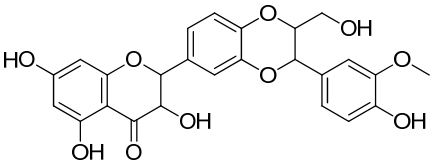
36		IN00047	253.23	19
37		IN00048	188.23	19
38		IN00049	432.38	42
39		IN00050	399.44	9
40		IN00051	286.08	11
41		IN00052	178.14	No inhibition
42		IN00053	624.59	No inhibition
43		IN00054	260.33	No inhibition

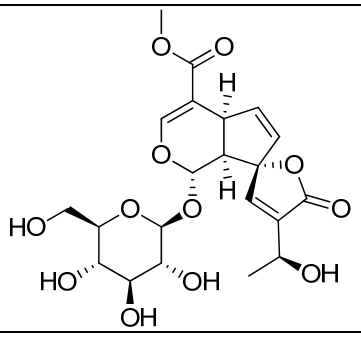
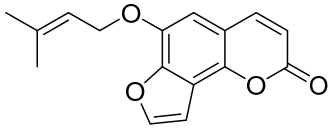
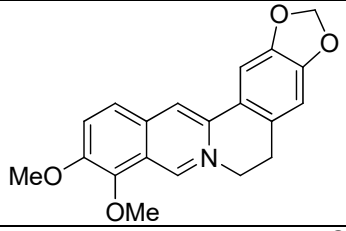
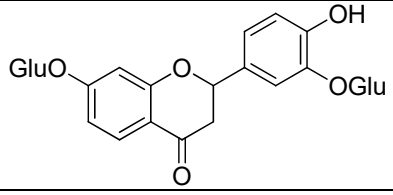
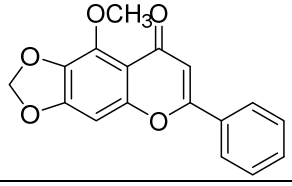
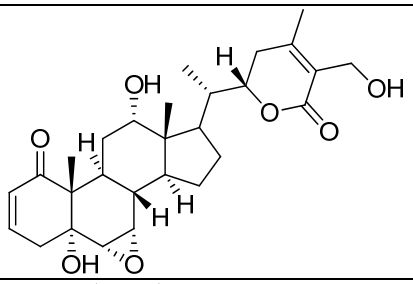
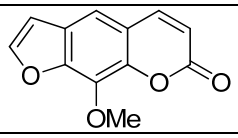
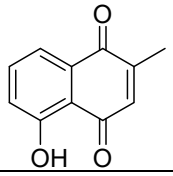
44		IN00055	610.52	7.7
45		IN00056	448.38	No inhibition
46		IN00057	192.26	No inhibition
47		IN00068	248.36	6.2
48		IN00069	348.0	70.7
49		IN00070	488.58	No inhibition
50		IN00071	174.20	8

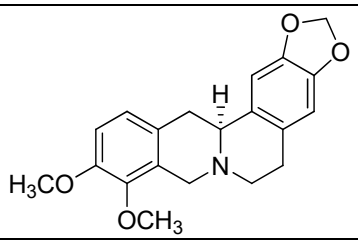
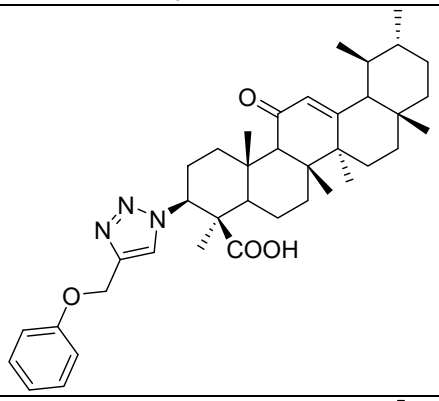
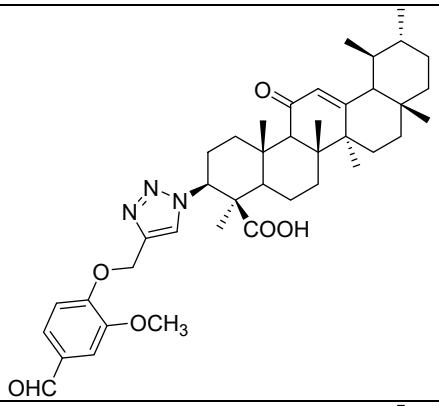
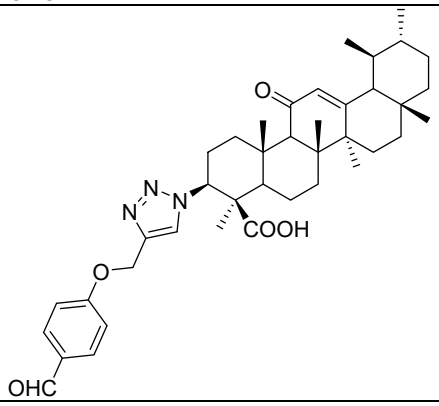
51		IN00072	178.19	No inhibition
52		IN00073	174.20	7
53		IN00074	192.21	2
54		IN00075	194.19	No inhibition
55		IN00076	192.21	No inhibition
56		IN00077	192.21	No inhibition
57		IN00078	174.20	No inhibition
58		IN00079	192.21	5
59		IN00080	174.20	No inhibition

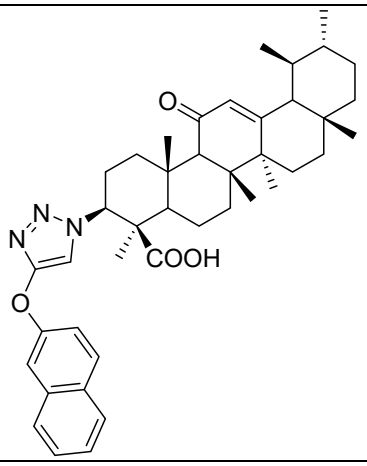
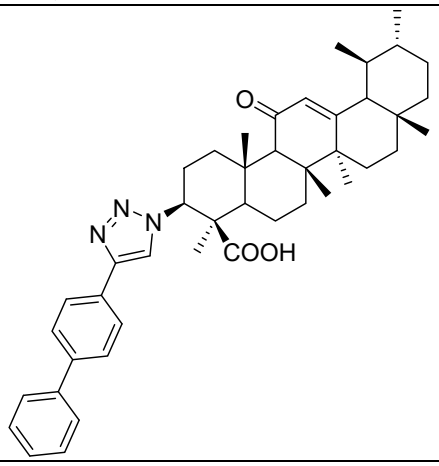
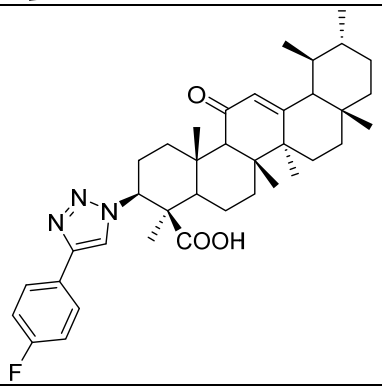
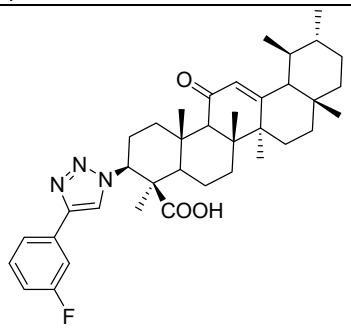
60		IN00081	202.16	20
61		IN00082	272.25	9
62		IN00083	197.2	No inhibition
63	 Racemic form	IN00084	286.28	56
64		IN00085	228.24	3
65		IN00086	385.41	1.4
66	 Sphondin	IN00087	216.19	67
67		IN00088	216.19	68
68		IN00089	246.30	No inhibition

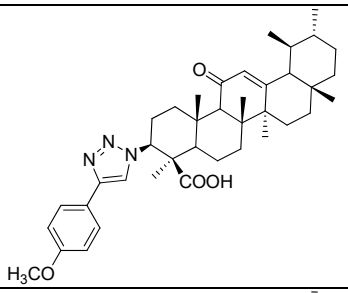
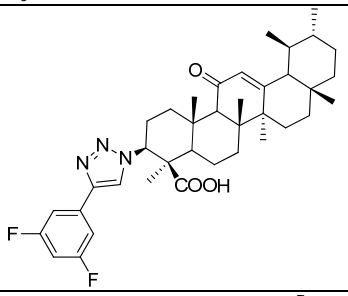
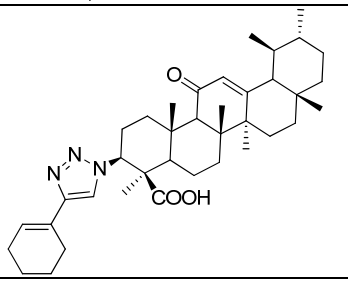
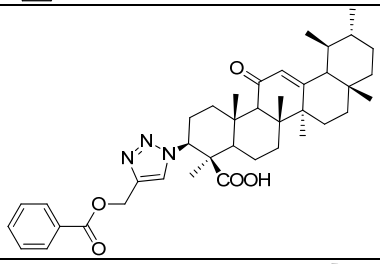
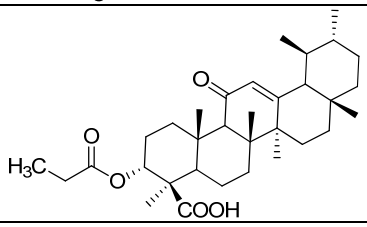
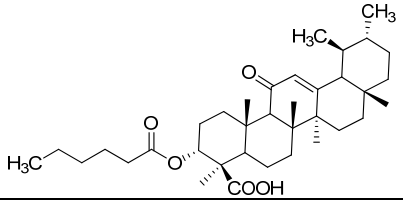
69		IN00090	422.34	No inhibition
70		IN00091	352.43	3
71		IN00092	620.8	30
72		IN00093	426.49	2.6
73		IN00094	355.43	No inhibition
74		IN00095	176.21	No inhibition
75		IN00096	252.69	No inhibition
76		IN00097	278.30	No inhibition
77		IN00098	250.29	2

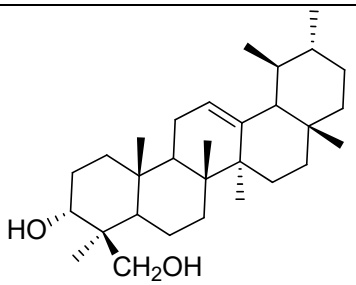
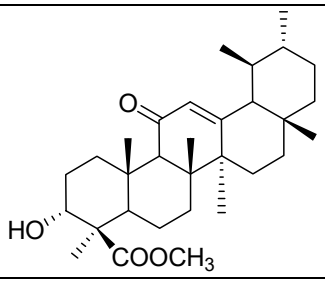
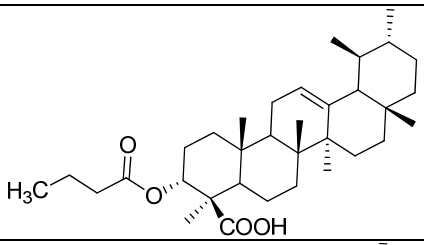
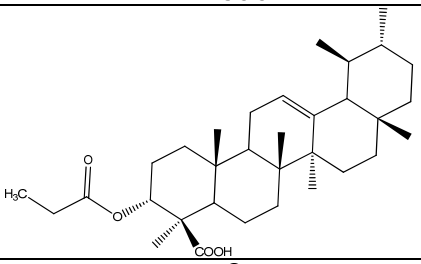
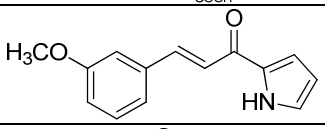
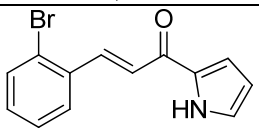
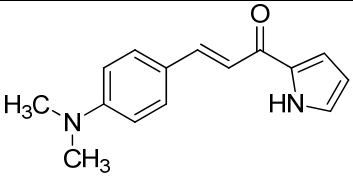
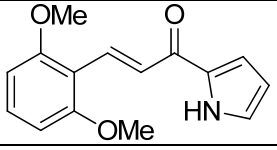
78		IN00099	450.44	No inhibition
79		IN00100	494.49	No inhibition
80		IN00101	286.24	30
81		IN00102	285.34	44
82		IN00103	312.32	20
83		IN00104	332.39	2
84		IN00105	164.16	No inhibition
85	 Racemic form	IN00106	482.44	5

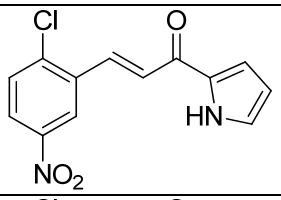
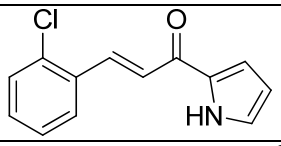
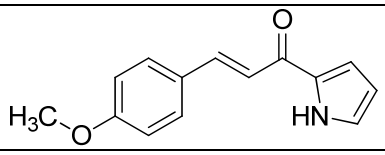
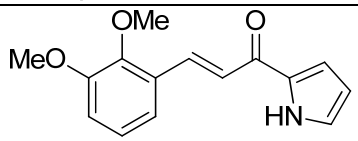
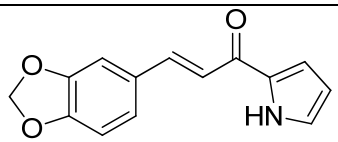
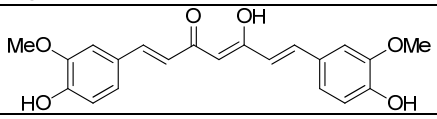
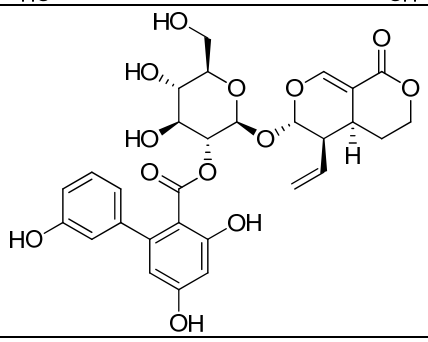
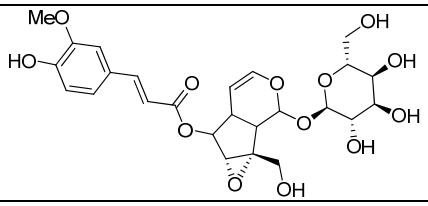
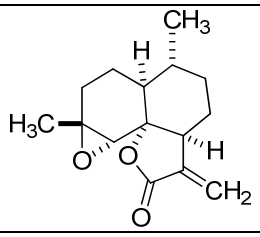
86		IN00107	470.42	No inhibition
87		IN00108	270.28	7
88		IN00109	372.82	48
89		IN00110	384.36	No inhibition
90		IN00111	296.27	41
91		IN00112	486.60	No inhibition
92		IN00113	216.19	84
93		IN00114	188.18	48

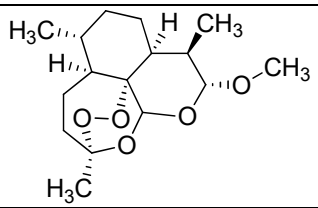
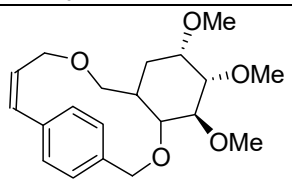
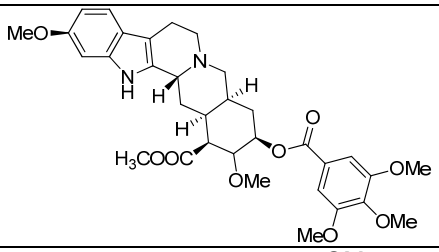
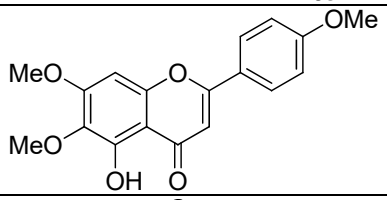
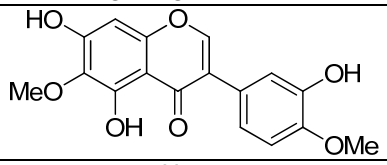
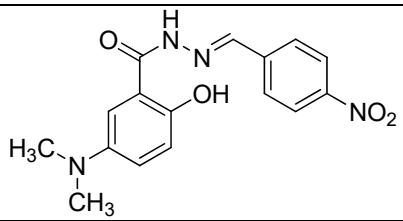
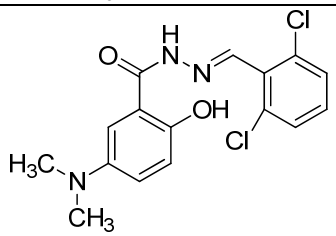
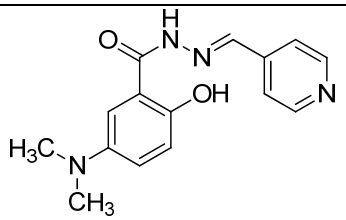
94		IN00115	339.34	No inhibition
95		IN00116	627.86	No inhibition
96		IN00117	685.89	No inhibition
97		IN00118	655.87	2

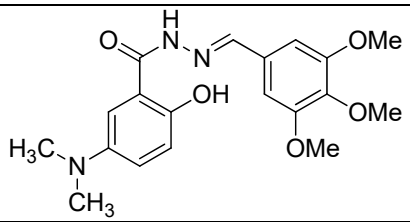
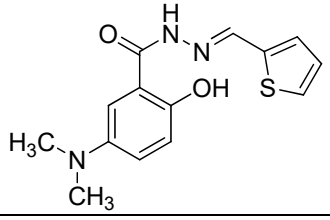
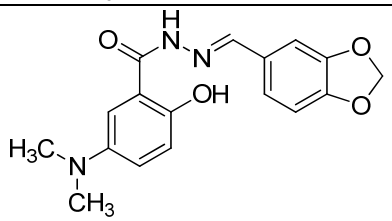
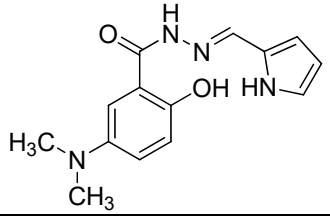
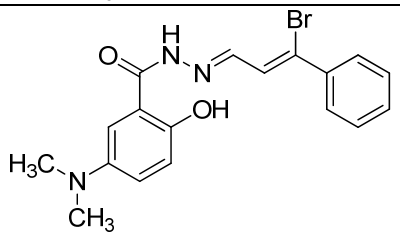
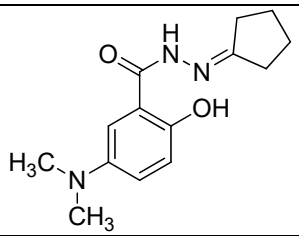
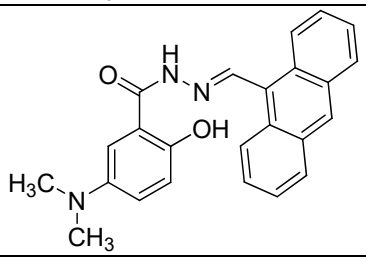
98		IN00119	663.89	4.8
99		IN00120	673.93	No inhibition
100		IN00121	615.82	2
101		IN00122	615.82	No inhibition

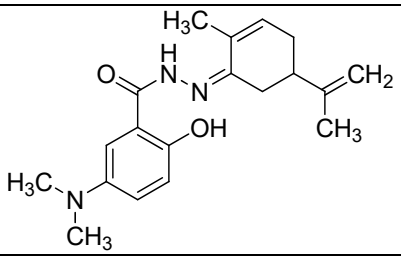
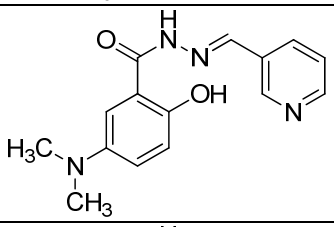
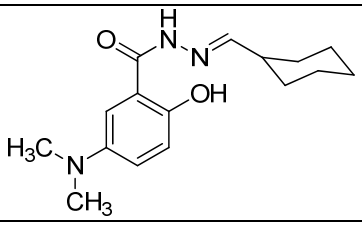
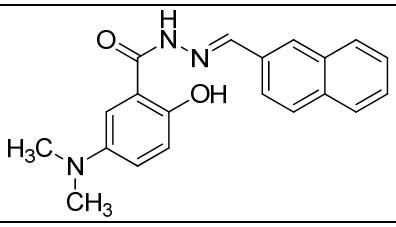
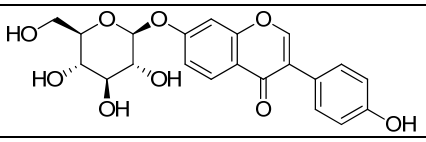
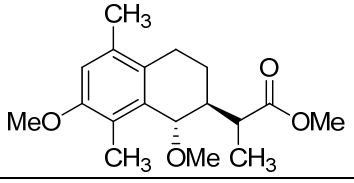
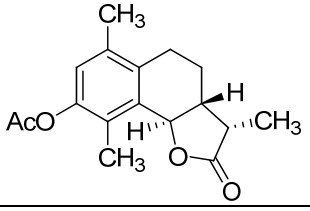
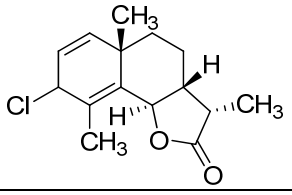
102		IN00123	627.86	No inhibition
103		IN00124	633.81	No inhibition
104		IN00125	601.86	6
104		IN00126	655.87	2
105		IN00127	526.75	4
106		IN00128	568.83	3

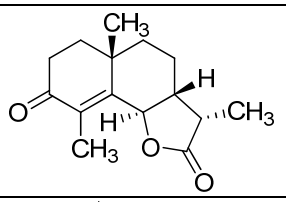
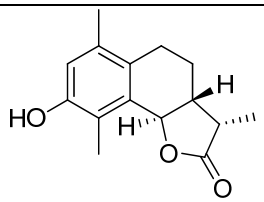
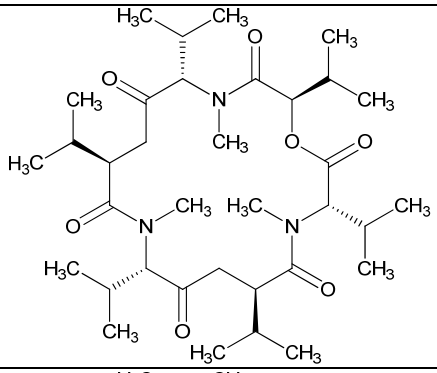
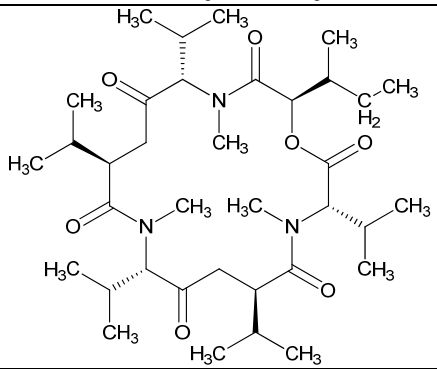
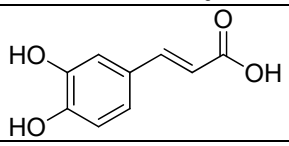
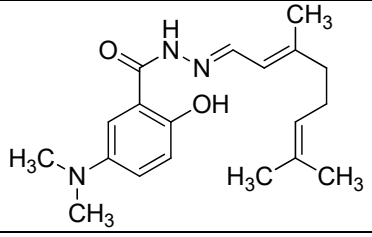
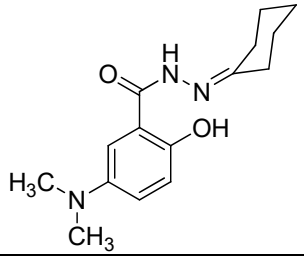
107		IN00129	442.72	No inhibition
108		IN000130	484.36	No inhibition
109		IN00131	526.79	No inhibition
110		IN00132	512.76	1
111		IN00133	227.26	70
112		IN00134	276.13	55
113		IN00135	240.30	7
114		IN00136	257.28	83

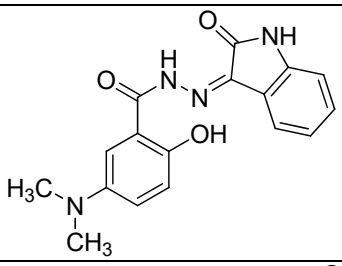
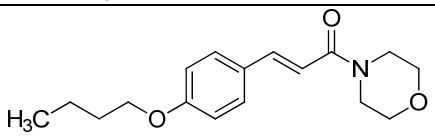
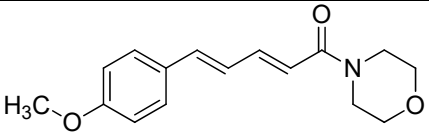
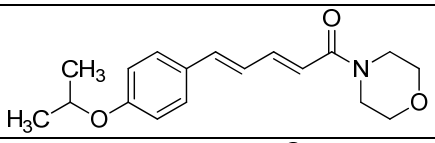
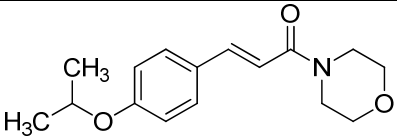
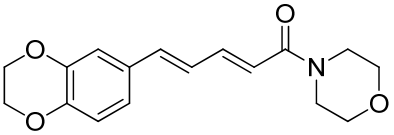
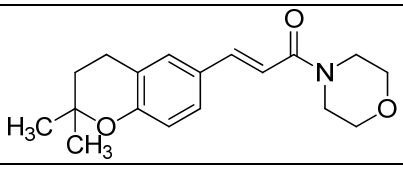
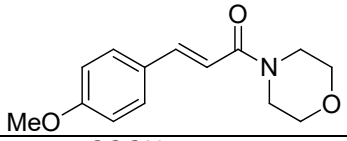
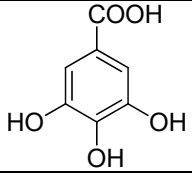
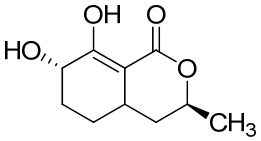
115		IN00137	277.68	15
116		IN00138	231.68	34
117		IN00139	227.26	25
118		IN00140	257.28	66
119		IN00141	241.24	93
120		IN00142	368.38	14
121		IN00144	584.57	No inhibition
122		IN00145	538.50	No inhibition
123		IN00146	248.32	4

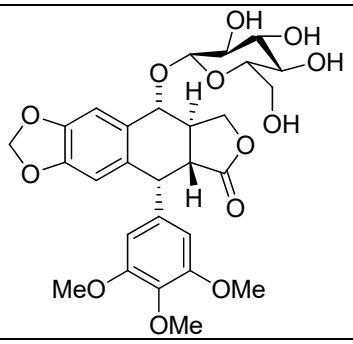
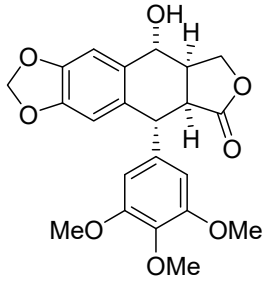
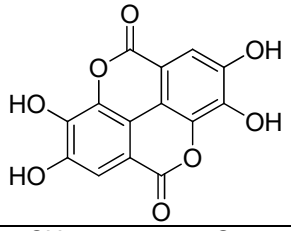
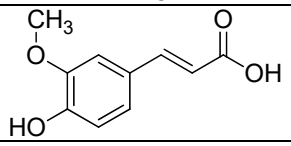
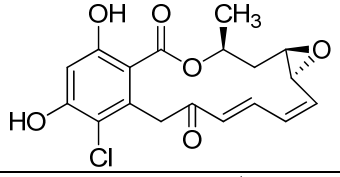
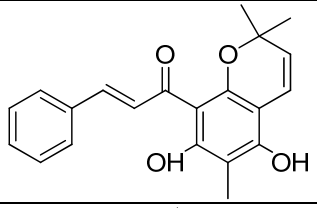
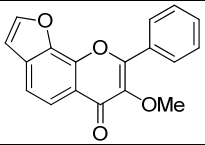
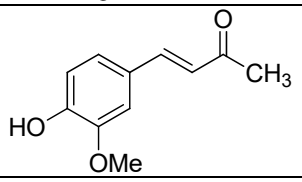
124		IN00147	298.37	No inhibition
125		IN00148	348.9	11.5
126		IN00149	608.68	3.2
127		IN00150	328.32	27
128		IN00151	330.29	No inhibition
129		IN00152	329.33	37.5
130		IN00153	352.22	69
131		IN00154	284.31	76

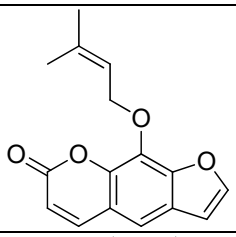
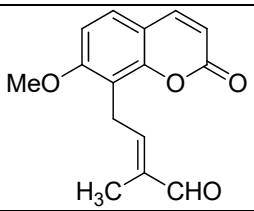
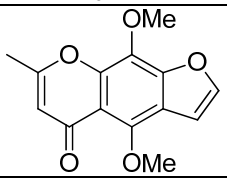
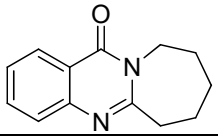
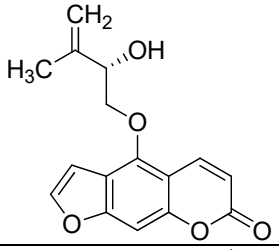
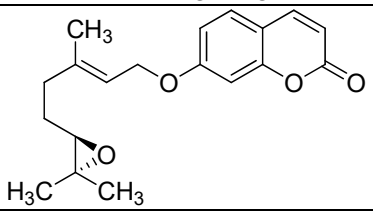
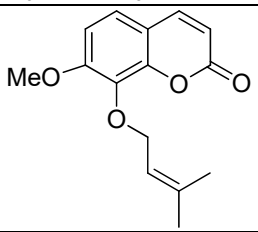
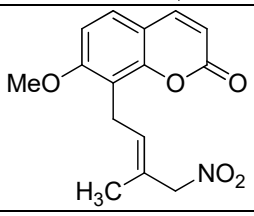
132		IN00155	373.40	57
133		IN00156	289.35	65
134		IN00157	327.33	12
135		IN00158	272.30	80
136		IN00159	388.26	76
137		IN00160	261.32	48
138		IN00161	383.44	35

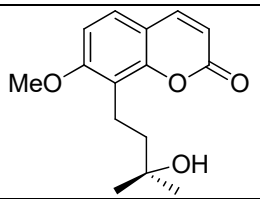
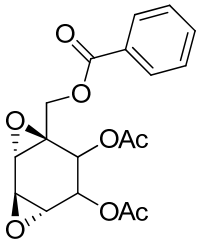
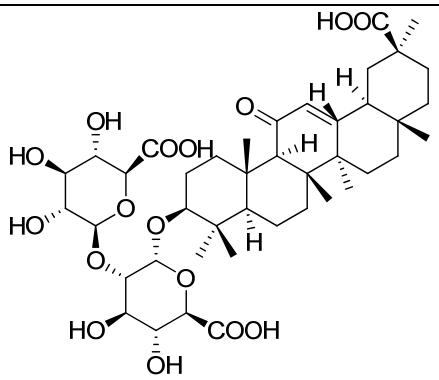
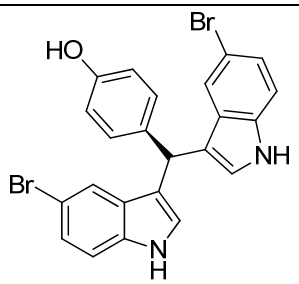
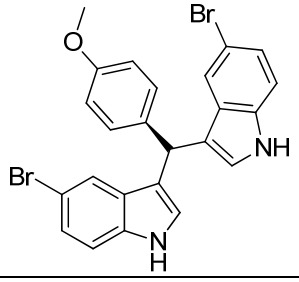
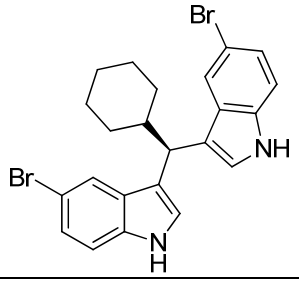
139		IN00162	327.42	56
140		IN00163	284.31	75
141		IN00164	289.37	36
142		IN00165	333.38	7
143		IN00166	416.38	No inhibition
144		IN00167	306.40	No inhibition
145		IN00168	288.34	No inhibition
146		IN00169	266.76	No inhibition

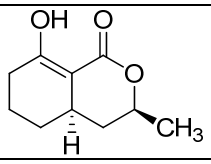
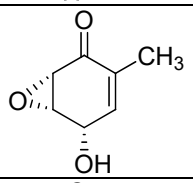
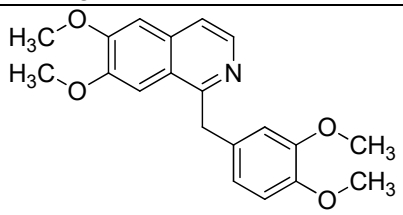
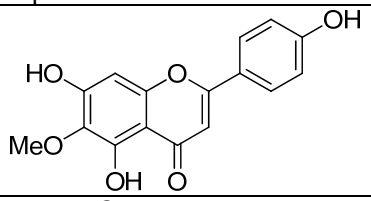
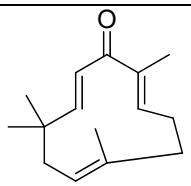
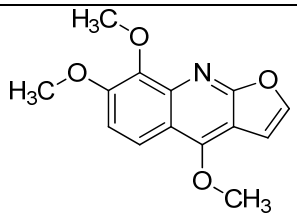
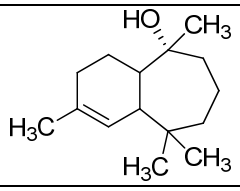
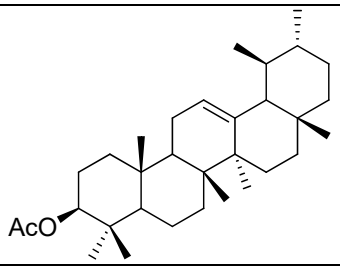
147		IN00170	248.32	No inhibition
148		IN00171	246.30	No inhibition
149		IN00173	635.87	No inhibition
150		IN00174	649.90	5.9
151		IN00175	180.16	No inhibition
152		IN00177	329.44	84
153		IN00178	275.35	28

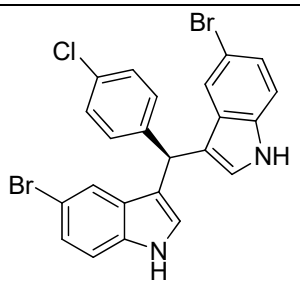
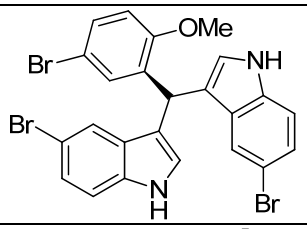
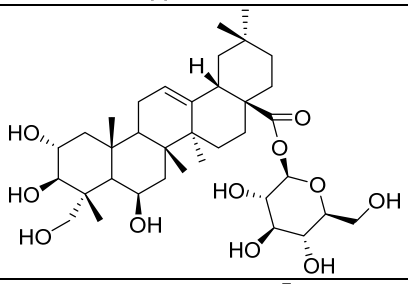
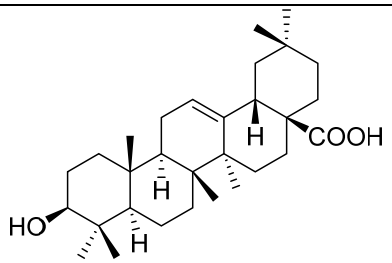
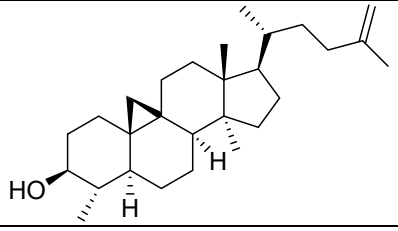
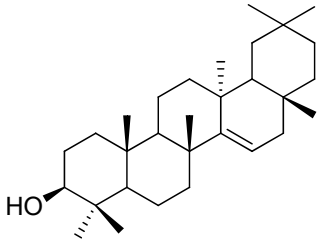
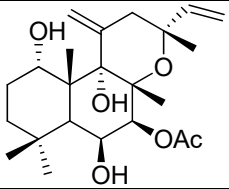
154		IN00179	324.33	35
155		IN00180	289.37	1
156		IN00181	273.33	10
157		IN00182	301.38	No inhibition
158		IN00183	275.34	7
159		IN00184	301.34	No inhibition
160		IN00185	301.38	4
161		IN00186	247.29	No inhibition
162		IN00187	170.12	45
163		IN00188	198.22	No inhibition

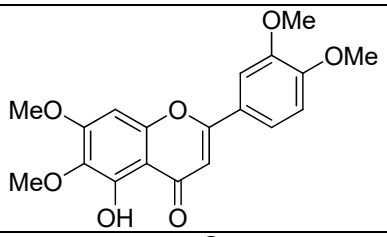
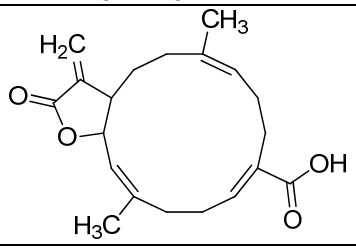
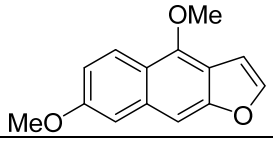
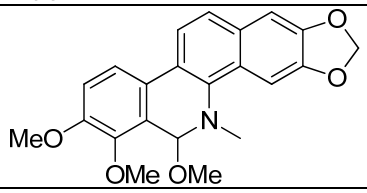
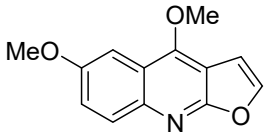
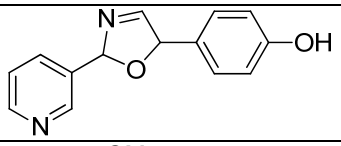
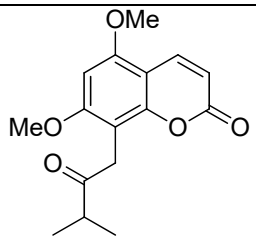
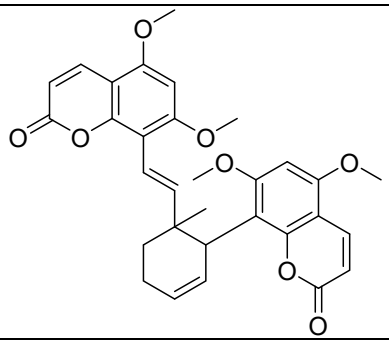
164		IN00189	576.55	No inhibition
165	 Picropodophyllotoxin	IN00190	414.41	2
166		IN00191	302.19	No inhibition
167		IN00192	194.18	No inhibition
168		IN00193	364.80	No inhibition
169		IN00194	338.40	52
170		IN00195	294.30	97
170		IN00196	192.21	2

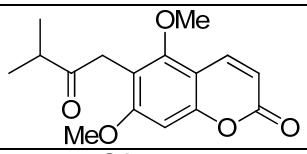
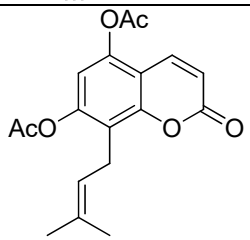
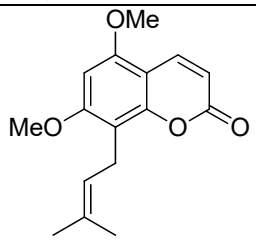
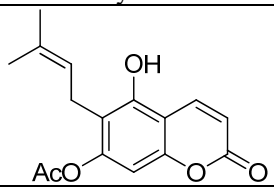
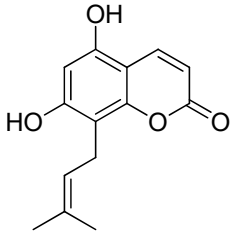
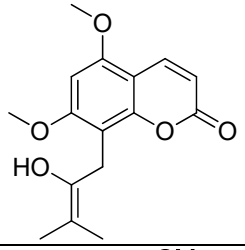
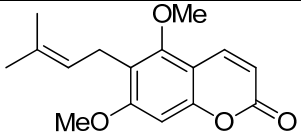
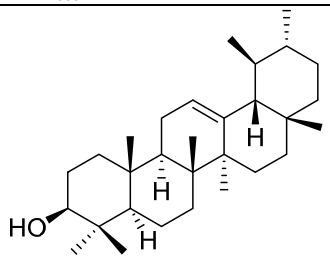
171		IN00197	270.28	71.4
172		IN00198	258.27	7
173		IN00199	302.45	92
174		IN00200	214.26	No inhibition
175		IN00201	286.28	No inhibition
176		IN00202	314.38	22
17		IN00203	244.29	33
177		IN00204	290.29	15

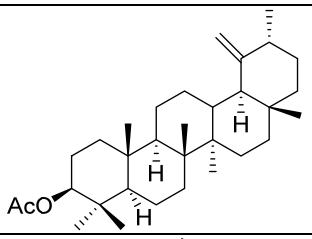
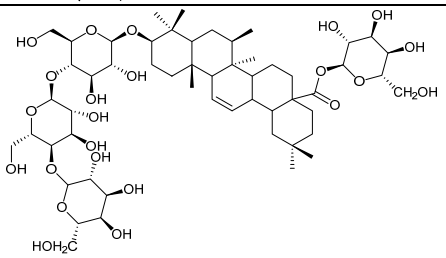
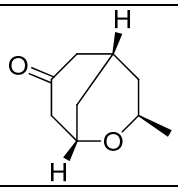
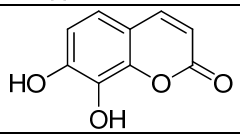
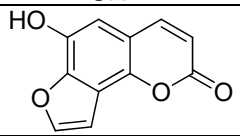
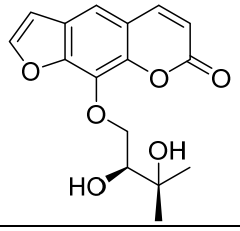
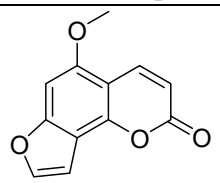
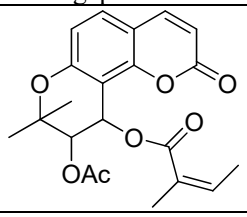
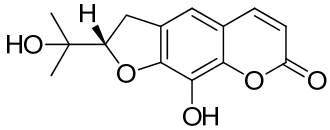
178		IN00205	262.30	18.2
179		IN00206	362.33	No inhibition
180		IN00207	822.93	No inhibition
181		IN00208	496.19	No inhibition
182		IN00209	540.25	No inhibition
183		IN00210	486.24	No inhibition

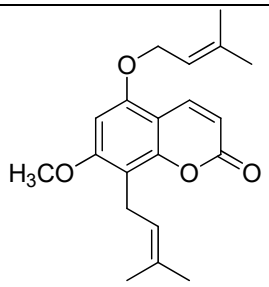
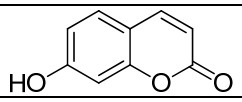
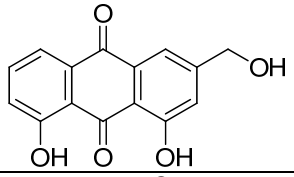
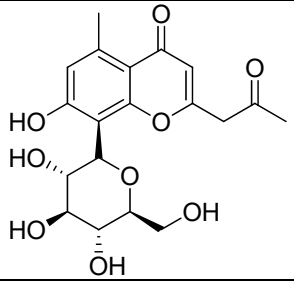
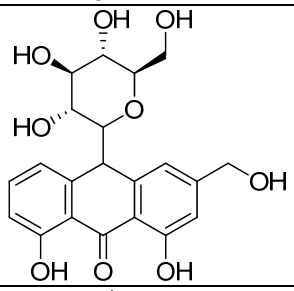
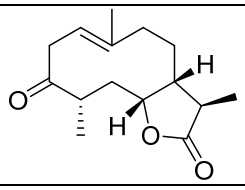
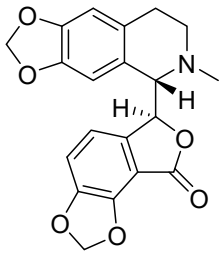
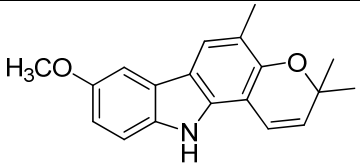
184		IN00211	182.22	No inhibition
185		IN00212	140.14	No inhibition
186	 Papaverine	IN00213	339.39	9.5
187		IN00214	300.26	86
188		IN00215	218.33	No inhibition
189		IN00216	259.26	27
190		IN00217	222.37	11.3
191		IN00218	468.75	No inhibition

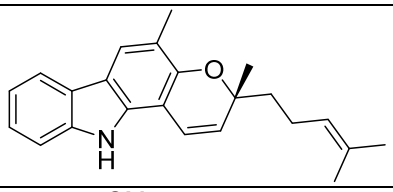
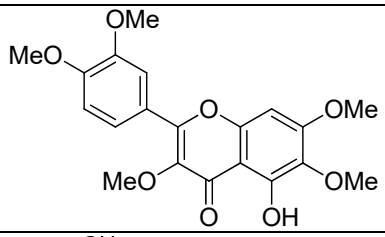
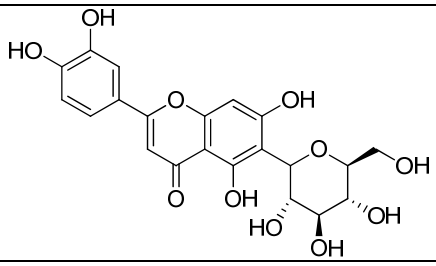
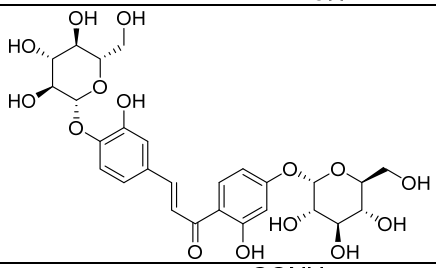
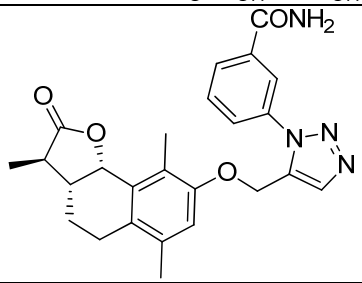
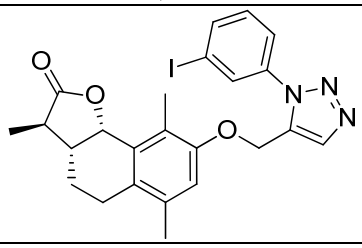
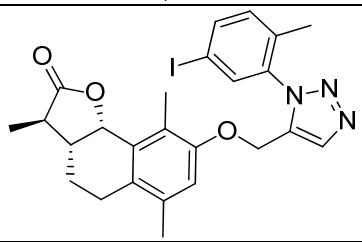
192		IN00219	514.64	3
193		IN00220	589.12	No inhibition
194		IN00221	633.81	No inhibition
195		IN00222	456.70	2.8
196		IN00223	398.66	2
197		IN00224	426.72	7
198		IN00225	408.53	No inhibition

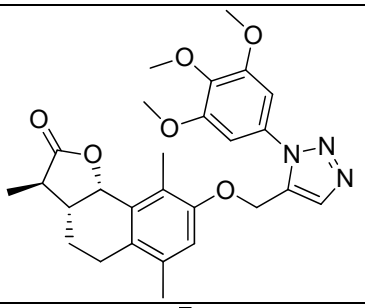
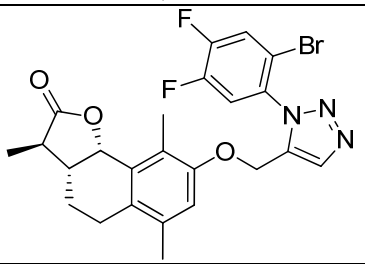
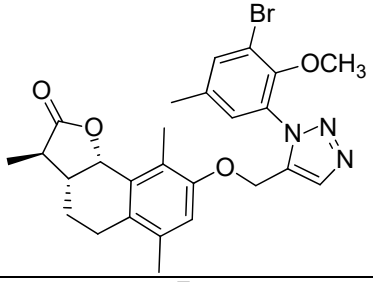
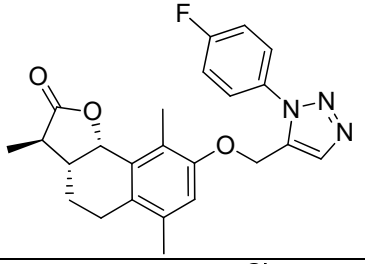
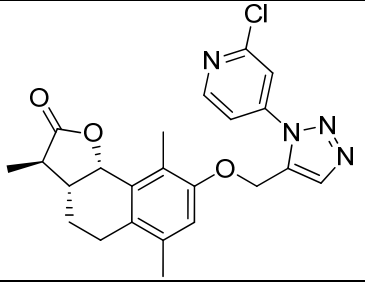
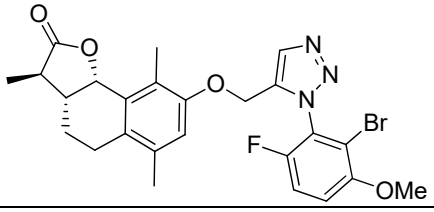
199		IN00226	360.36	80
200		IN00227	330.42	No inhibition
201		IN00228	231.25	No inhibition
202		IN00229	379.41	90
203		IN00230	229.25	17
204		IN00231	240.26	No inhibition
205		IN00232	290.31	No inhibition
206		IN00233	544.59	No inhibition

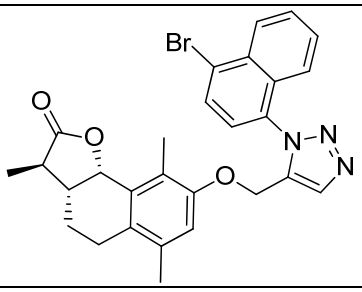
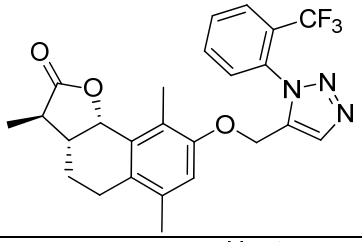
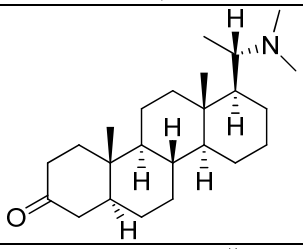
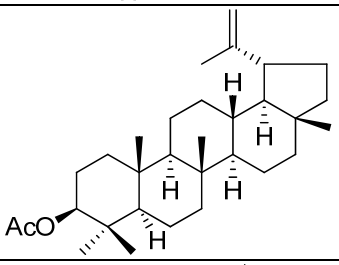
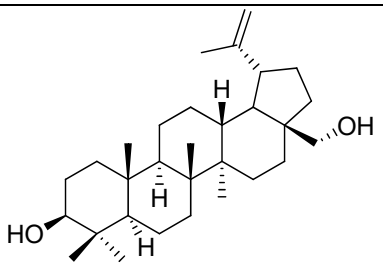
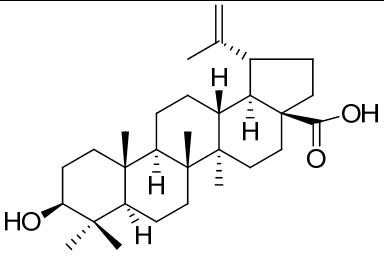
207		IN00234	290.31	No inhibition
208		IN00235	330.33	3.7
209[c]	 Coumurrayin	IN00236	274.31	3
210		IN00237	288.30	No inhibition
211		IN00238	246.26	17
212		IN00239	290.31	No inhibition
213		IN00240	274.31	6.4
214		IN00241	426.72	No inhibition

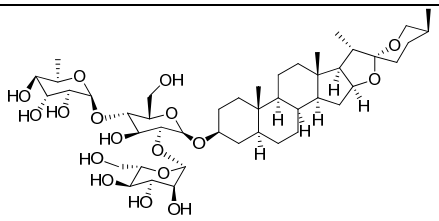
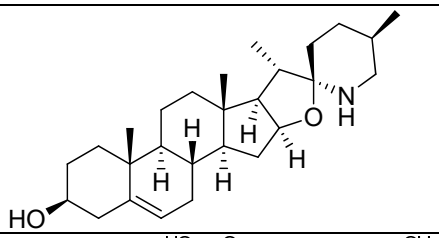
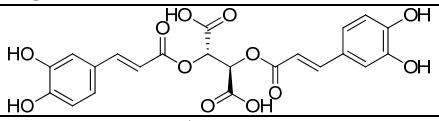
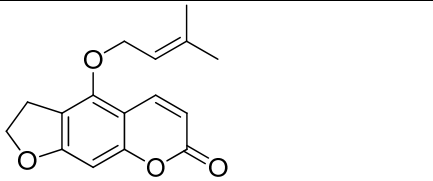
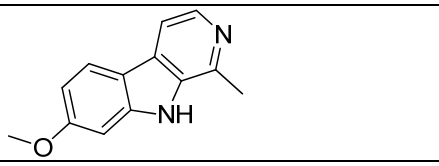
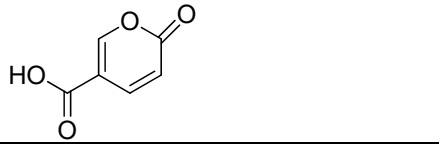
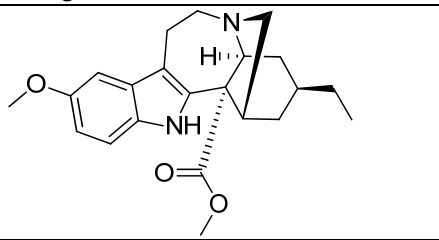
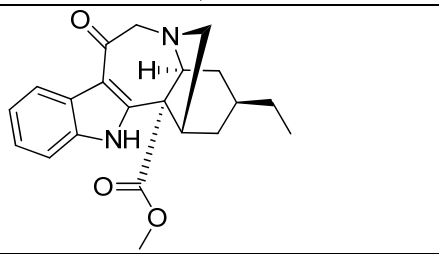
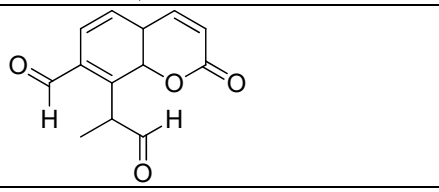
215		IN00242	468.75	No inhibition
216		IN00243	1105.26	No inhibition
217		IN00244	154.21	No inhibition
218		IN00245	178.14	No inhibition
219		IN00246	202.16	No inhibition
220		IN00247	304.29	72
221	 Isobergapten	IN00248	216.19	No inhibition
222		IN00249	386.40	1
223		IN00250	262.26	No inhibition

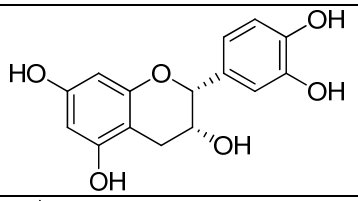
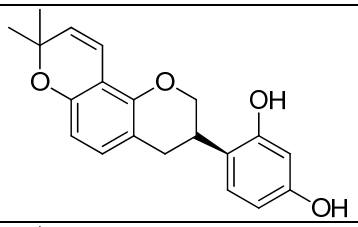
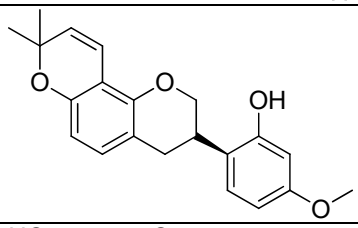
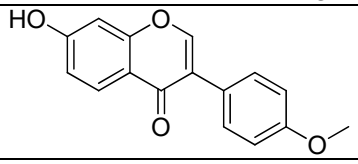
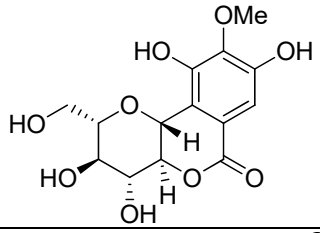
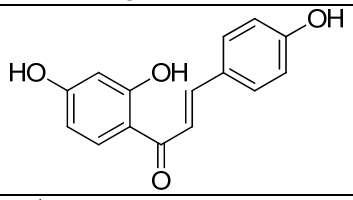
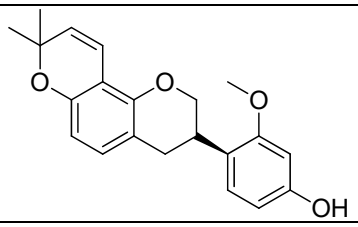
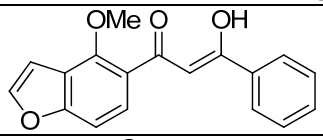
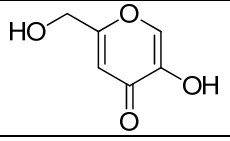
224	 Sesibiricin	IN00251	328.40	1.6
225		IN00252	162.14	5
226		IN00253	270.24	16
227		IN00254	394.37	No inhibition
228		IN00255	418.39	No inhibition
229		IN00256	250.33	No inhibition
230		IN00257	367.35	7
231		IN00258	293.36	2

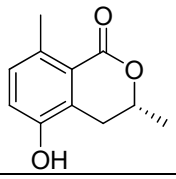
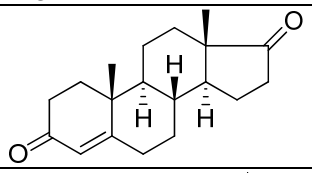
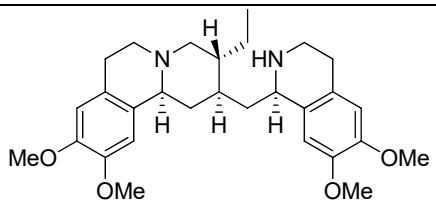
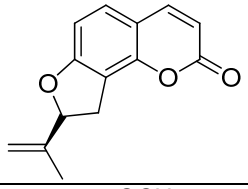
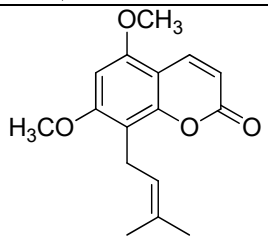
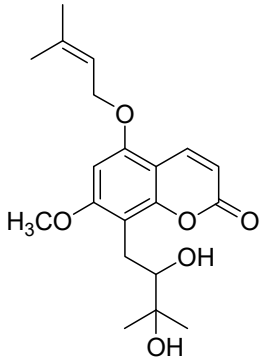
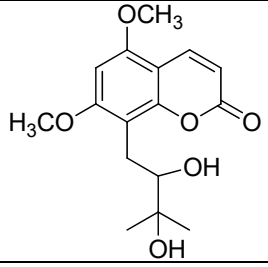
232		IN00259	331.45	10
233		IN00260	388.37	32
234		IN00261	448.38	No inhibition
235		IN00262	596.53	No inhibition
236		IN00263	446.50	No inhibition
237		IN00264	529.37	No inhibition
238		IN00265	543.40	No inhibition

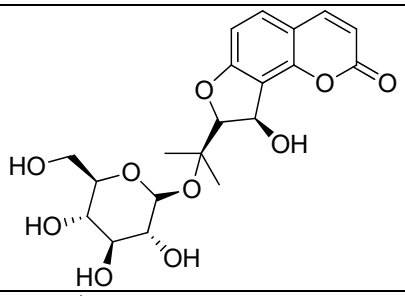
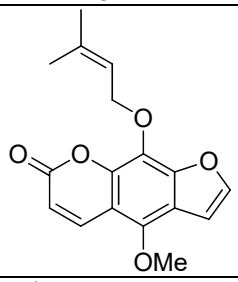
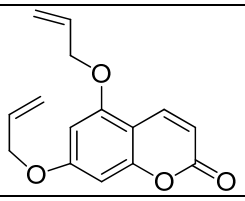
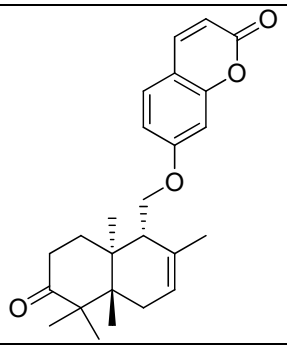
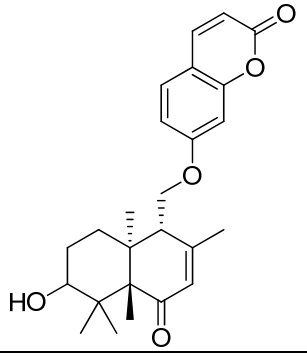
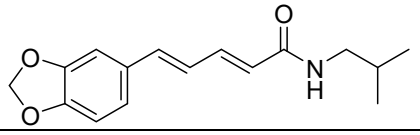
239		IN00266	493.55	No inhibition
240		IN00267	518.35	No inhibition
241		IN00268	526.42	No inhibition
242		IN00269	421.46	No inhibition
243		IN00270	438.91	1
244		IN00271	530.39	No inhibition

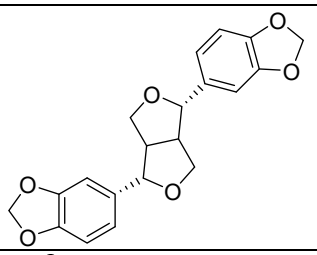
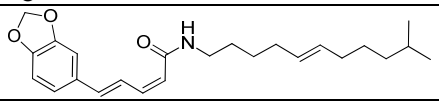
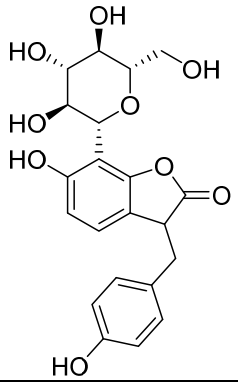
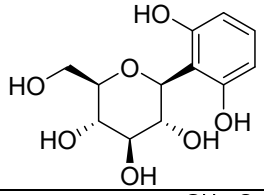
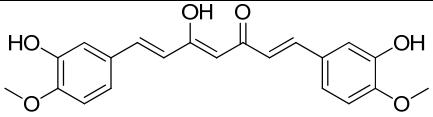
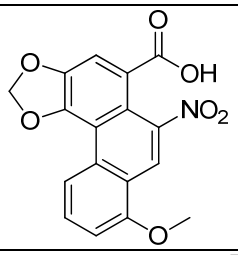
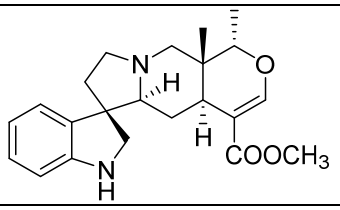
245		IN00272	532.43	No inhibition
246		IN00273	471.47	3
247		IN00276	359.59	No inhibition
248		IN00279	456.79	2.8
249[d]	 Betulin	IN00280	442.72	7
250[e]	 Betulinic acid	IN00281	456.70	0

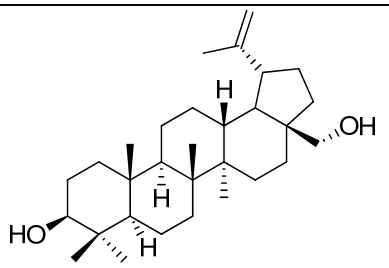
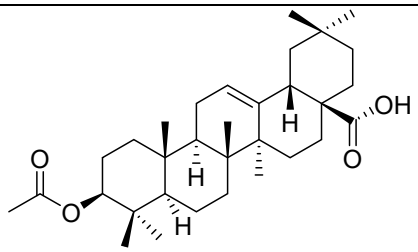
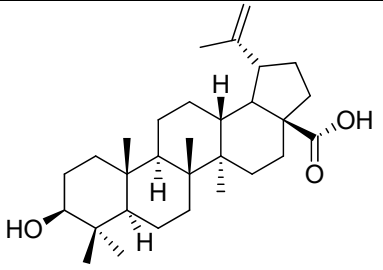
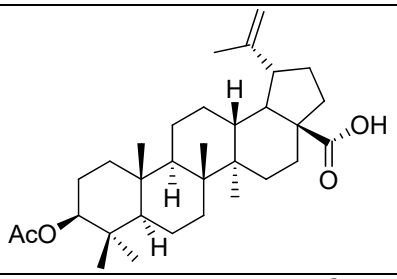
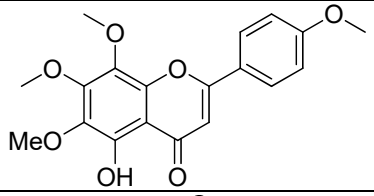
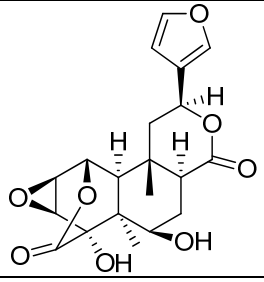
251		IN00282	889.03	No inhibition
252		IN00283	413.64	9.5
253		IN00284	474.37	16
254		IN00285	272.30	33
255		IN00286	248.71	No inhibition
256		IN00287	140.09	No inhibition
257		IN00288	368.47	No inhibition
258		IN00289	352.43	No inhibition
259		IN00290	232.23	No inhibition

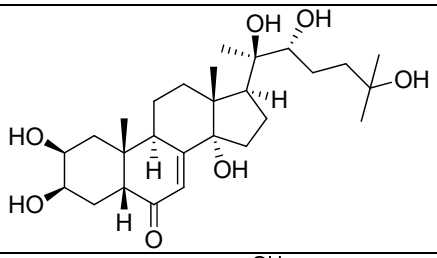
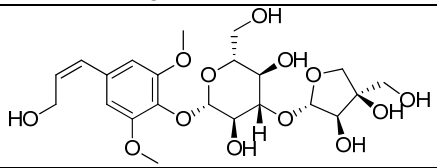
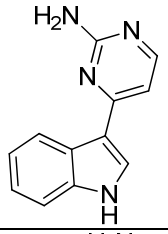
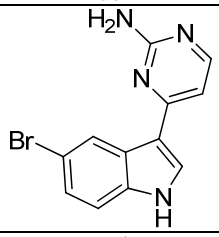
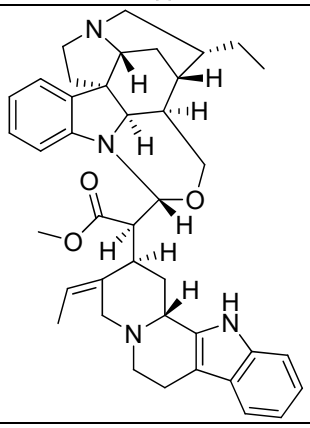
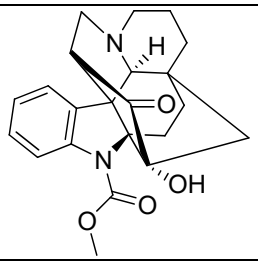
260		IN00292	290.27	No inhibition
261		IN00293	324.37	34
262		IN00294	338.40	No inhibition
263		IN00295	268.26	No inhibition
264		IN00296	328.27	No inhibition
265		IN00297	256.25	No inhibition
266		IN00298	338.40	No inhibition
267		IN00299	294.30	85
268		IN00300	142.11	No inhibition

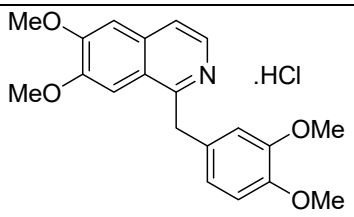
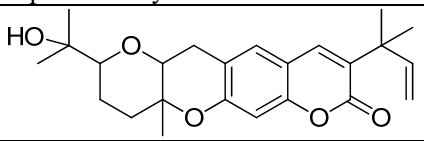
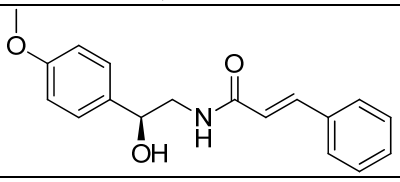
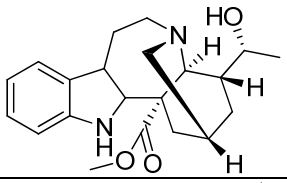
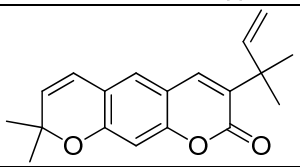
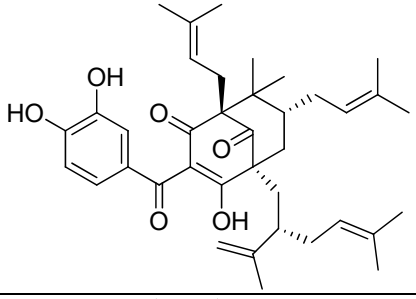
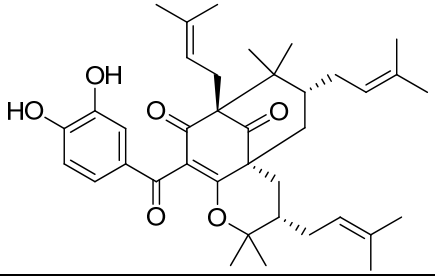
269		IN00301	192.21	No inhibition
270		IN00302	286.41	No inhibition
271	 2HCl	IN00303	553.56	No inhibition
272		IN00304	228.24	No inhibition
273 [c]	 Coumurrayin	IN00305	274.31	3
274	 Sesebrinol	IN00306	362.42	21
275		IN00307	308.33	No inhibition

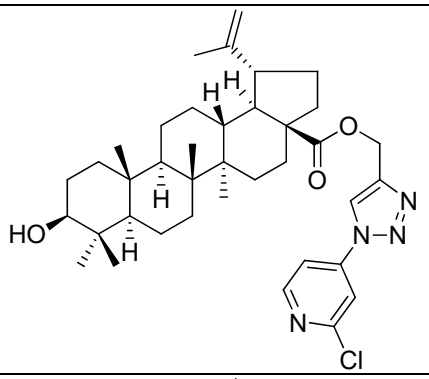
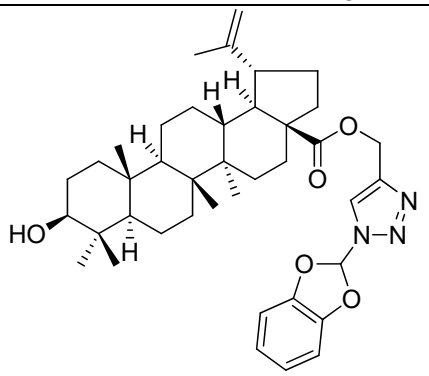
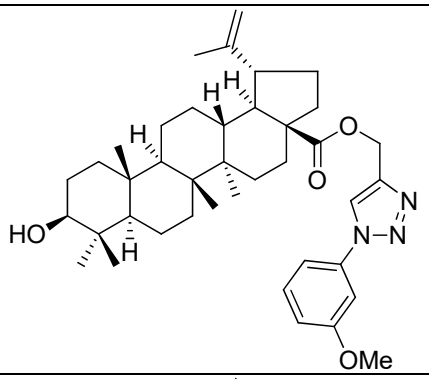
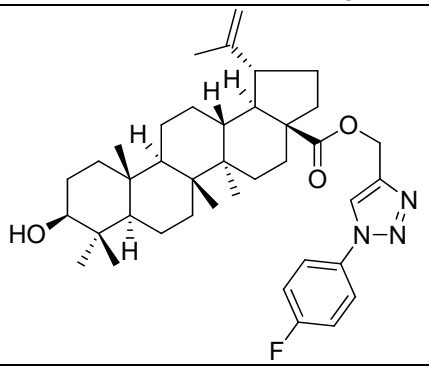
276		IN00308	424.40	No inhibition
277		IN00309	300.31	97
278		IN00310	258.27	69
279		IN00311	380.48	No inhibition
280		IN00312	410.12	No inhibition
281		IN00313	273.33	11

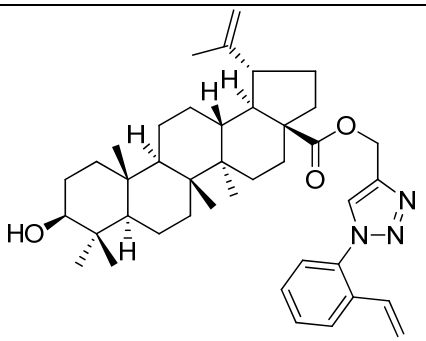
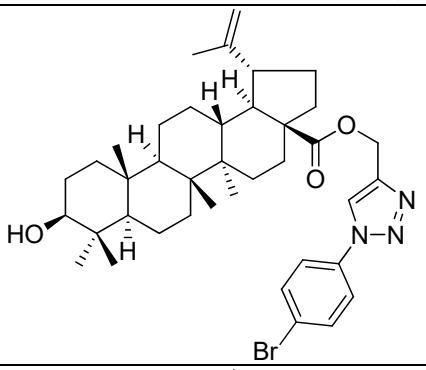
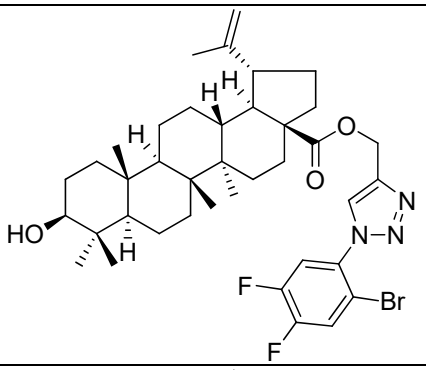
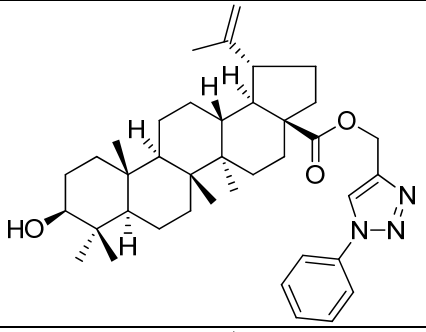
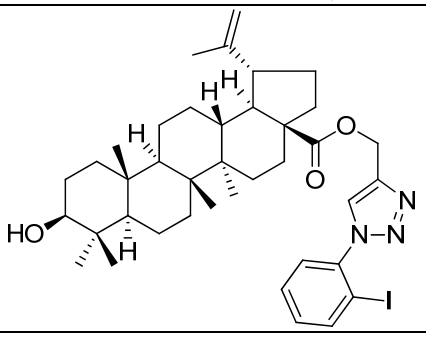
282		IN00314	354.35	No inhibition
283		IN00315	383.52	3
284		IN00316	416.42	No inhibition
285		IN00317	272.25	No inhibition
286		IN00318	370.40	No inhibition
287		IN00319	342.28	12
288		IN00321	368.43	No inhibition

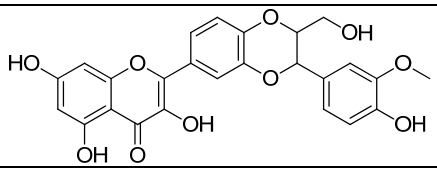
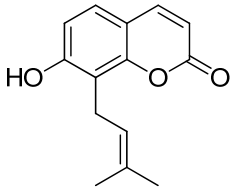
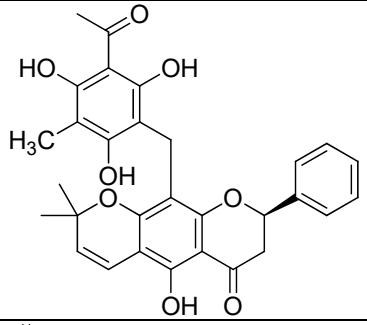
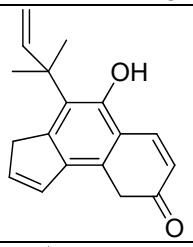
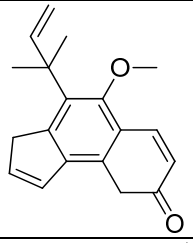
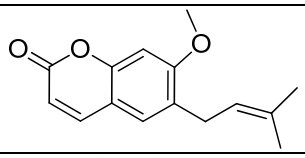
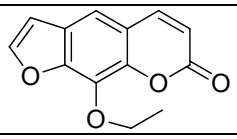
289[d]	 Betulin	IN00322	442.72	5.9
290		IN00323	496.76	No inhibition
291[e]	 Betulinic acid	IN00324	456.70	No inhibition
292		IN00325	498.74	No inhibition
293		IN00328	358.34	No inhibition
294		IN00329	390.38	No inhibition

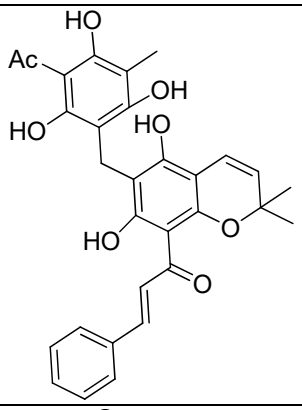
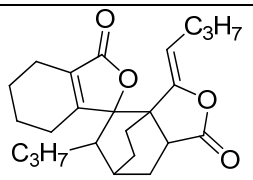
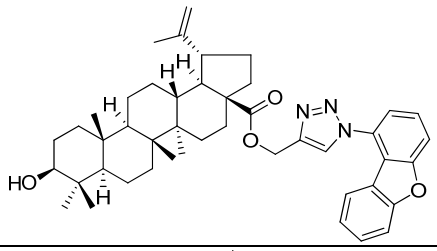
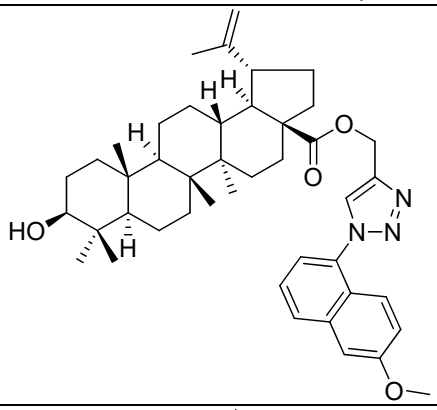
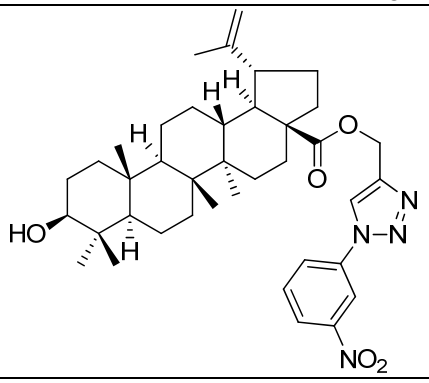
295		IN00330	480.63	3
296		IN00331	504.48	No inhibition
297		IN00332	210.23	38
298		IN00333	289.13	50
299		IN00336	632.83	No inhibition
300		IN00337	380.44	No inhibition

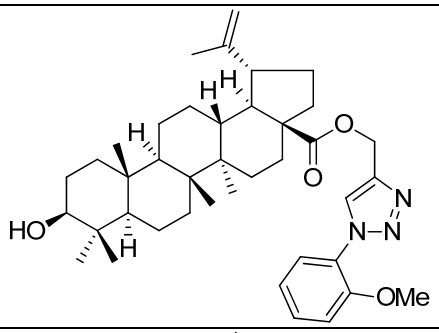
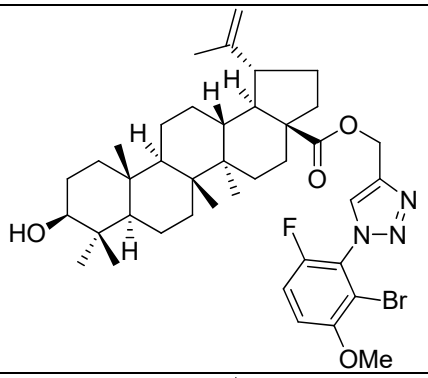
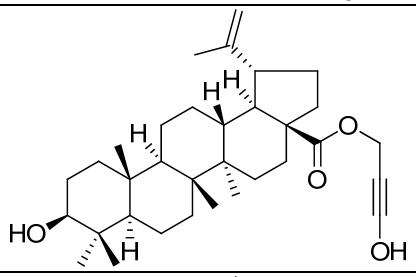
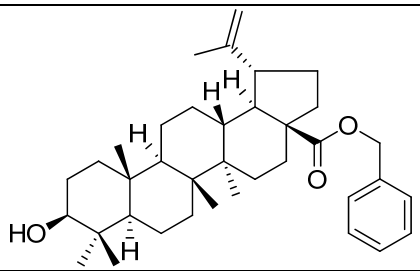
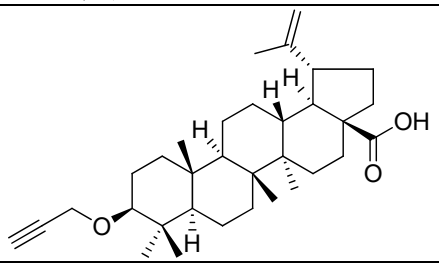
301	 Papaverine Hydrochloride	IN00338	375.12	9
302		IN00339	398.49	8
303		IN00340	297.35	No inhibition
304		IN00341	356.46	No inhibition
305		IN00343	228.24	28
306		IN00344	602.80	18
307		IN00345	602.80	10

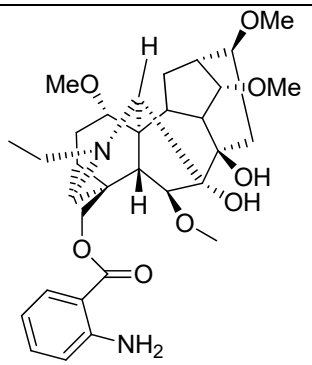
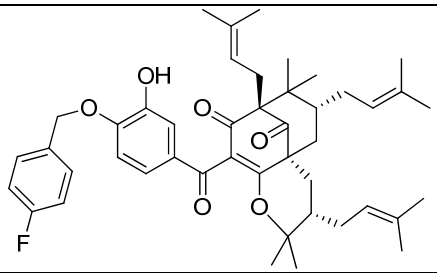
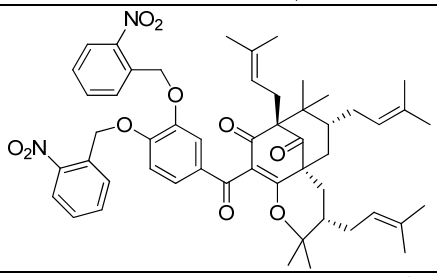
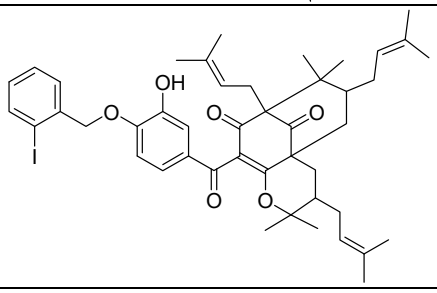
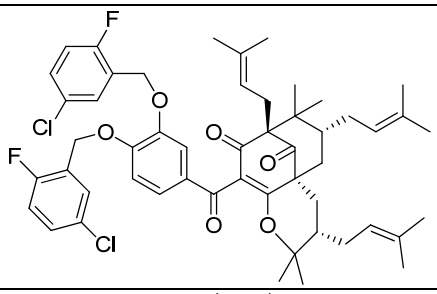
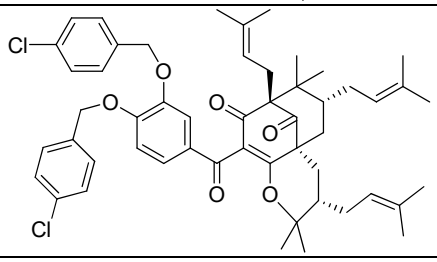
308		IN00346	649.31	No inhibition
309		IN00348	657.88	No inhibition
310		IN00349	643.90	No inhibition
311		IN00350	631.86	No inhibition

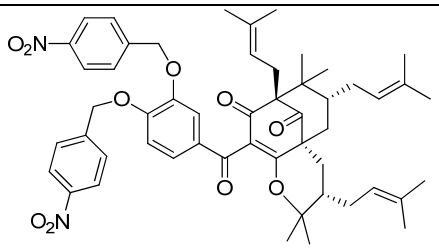
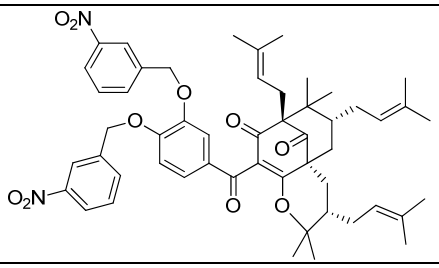
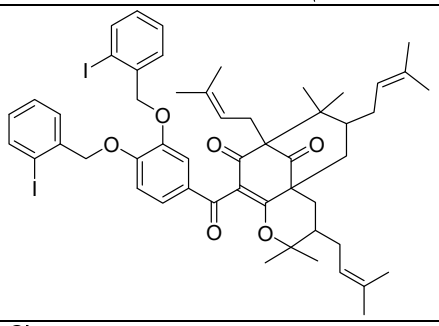
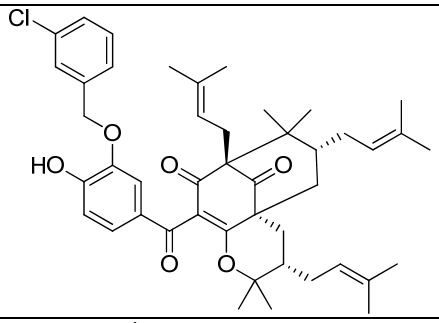
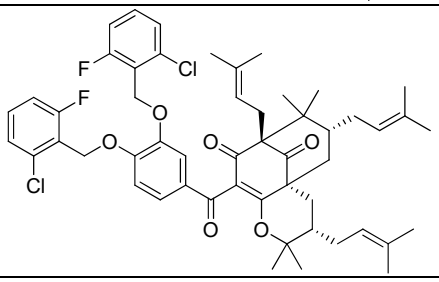
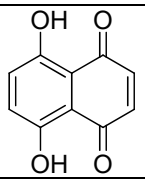
312		IN00351	639.91	No inhibition
313		IN00352	692.77	17.5
314		IN00353	728.75	No inhibition
315		IN00354	613.87	No inhibition
316		IN00355	739.77	No inhibition

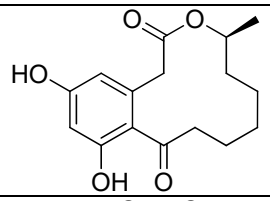
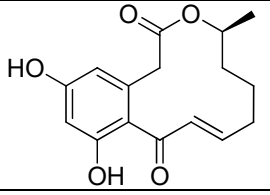
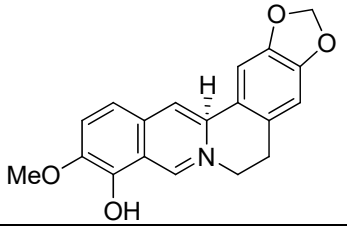
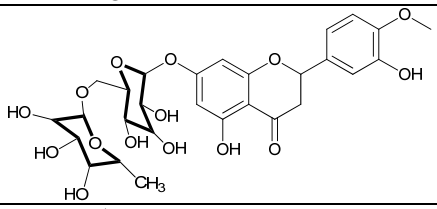
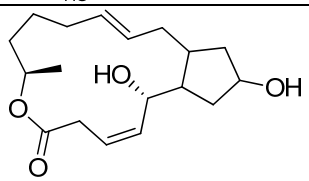
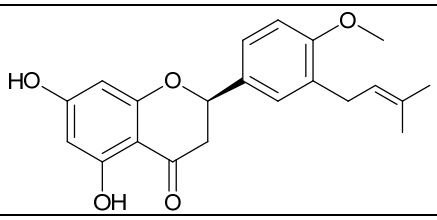
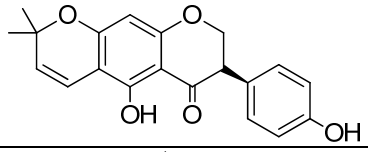
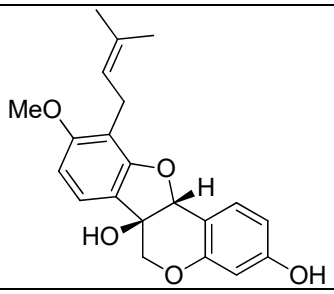
317		IN00357	478.45	36
318		IN00358	230.26	No inhibition
319		IN00359	516.54	No inhibition
320		IN00360	266.33	No inhibition
321		IN00361	280.36	No inhibition
322		IN00362	244.29	10
323		IN00363	230.22	89

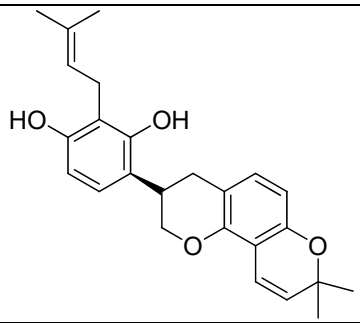
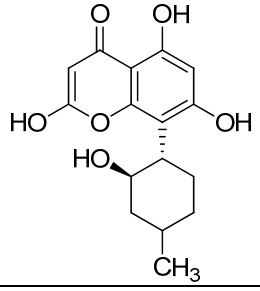
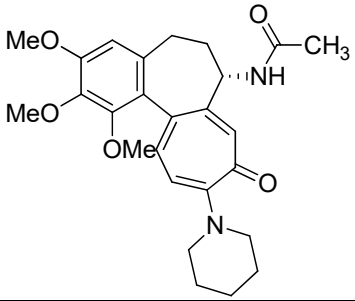
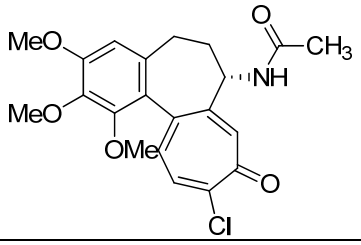
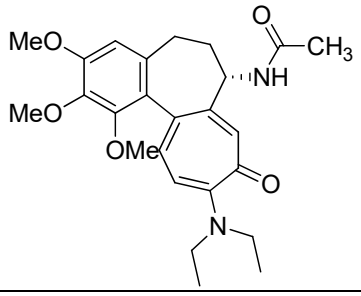
324		IN00364	516.54	27
325		IN00365	382.51	2
326		IN00369	703.95	3
327		IN00371	693.96	No inhibition
328		IN00372	659.88	No inhibition

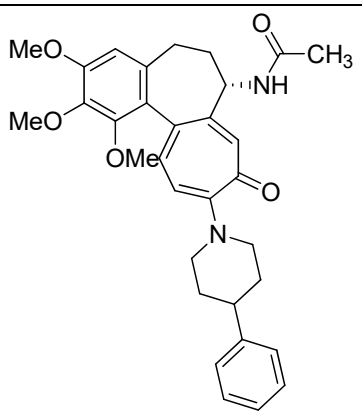
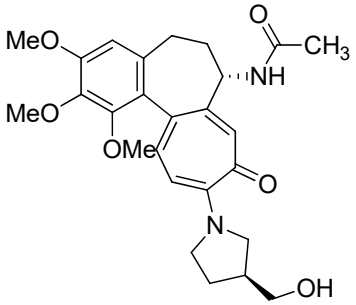
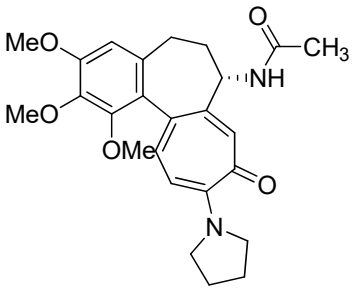
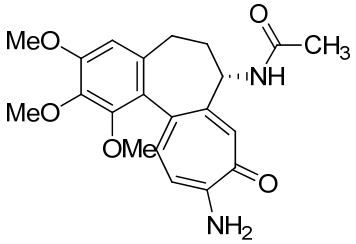
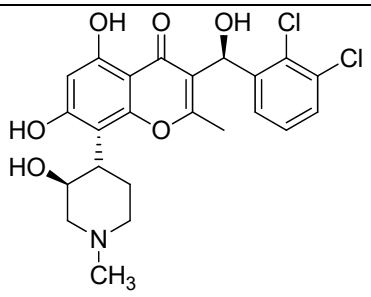
329		IN00373	643.90	No inhibition
330		IN00374	740.79	No inhibition
331		IN00375	495.75	No inhibition
332		IN00378	546.82	9.9
333		IN00380	494.75	No inhibition

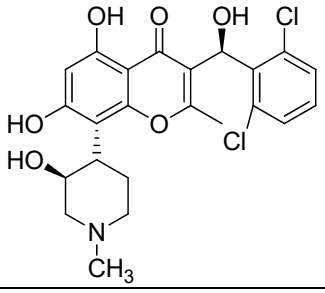
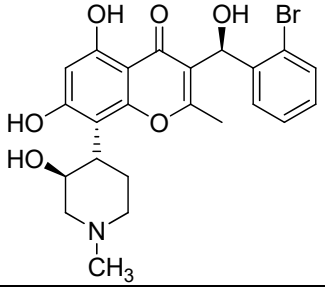
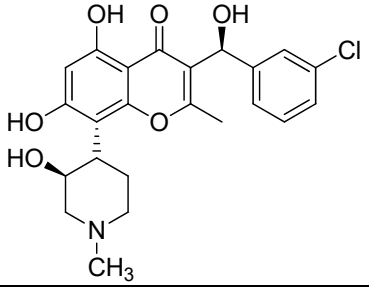
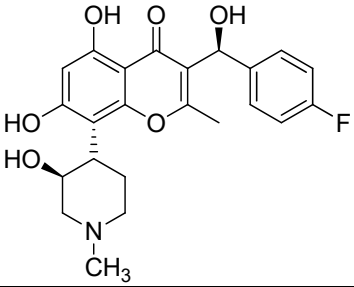
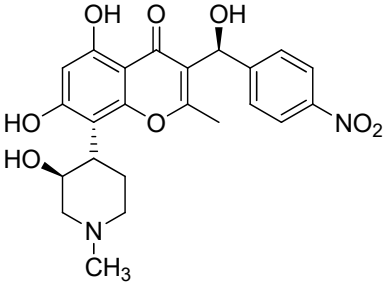
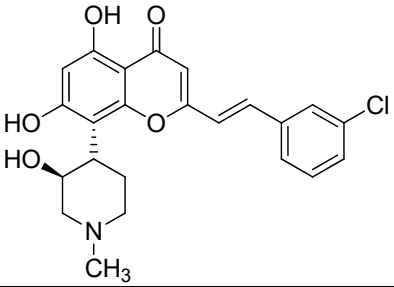
334		IN00386	586.72	1
335		IN00389	710.91	No inhibition
336		IN00390	875.06	No inhibition
337		IN00391	818.3	No inhibition
338		IN00392	887.82	No inhibition
339		IN00393	851.94	6.9

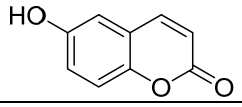
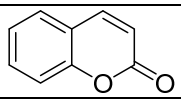
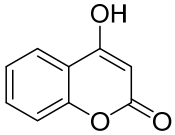
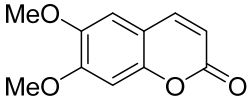
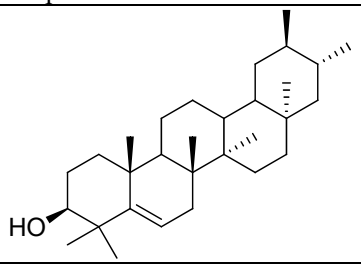
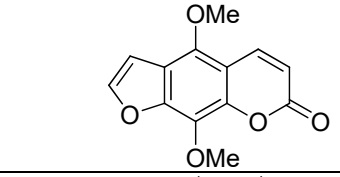
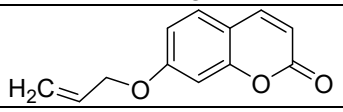
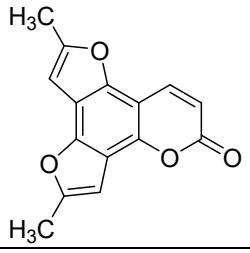
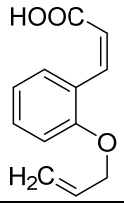
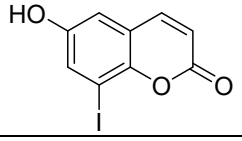
340		IN00394	875.6	13.1
341		IN00395	875.6	No inhibition
342		IN00397	1034.25	15.3
343		IN00399	727.37	No inhibition
344		IN00401	887.92	5.5
345		IN00416	190.15	99.6

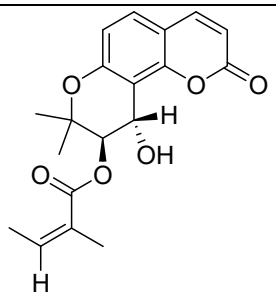
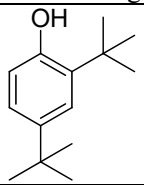
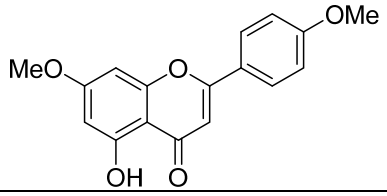
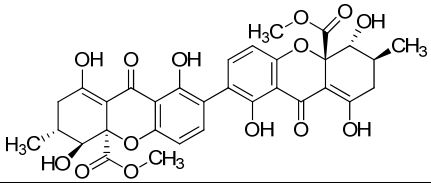
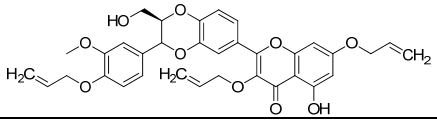
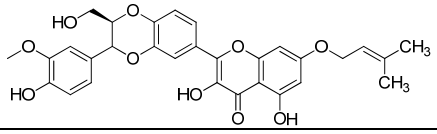
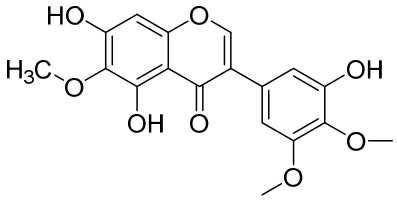
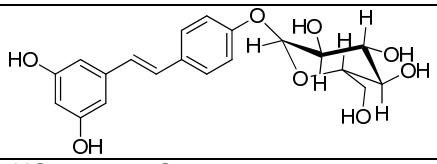
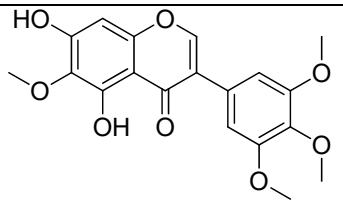
346		IN00417	292.33	2.7
347		IN00419	290.31	14.7
348		IN00420	322.33	24.7
349		IN00421	610.56	3.9
350		IN00423	280.36	8.9
351		IN00424	354.40	34.6
352		IN00425	336.34	14.9
353		IN00426	354.40	15

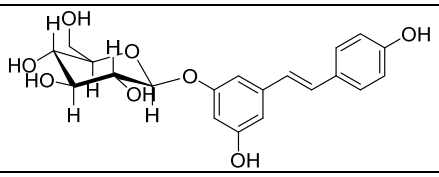
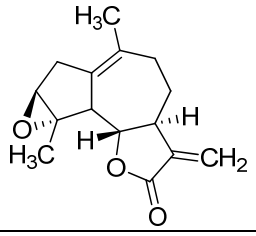
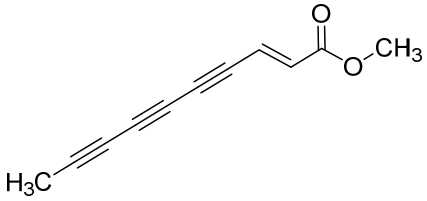
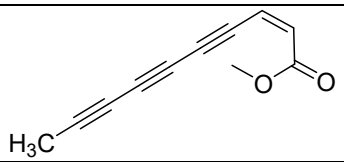
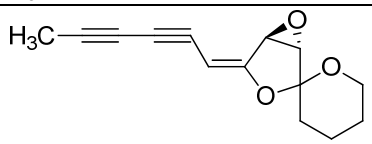
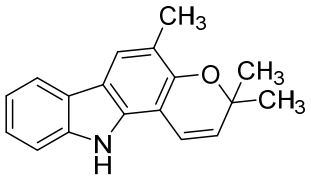
354		IN00431	392.49	24.9
355		IN00432	306.3	No inhibition
356		IN00436	452.54	8.3
357		IN00437	403.86	No inhibition
358		IN00438	440.53	3

359		IN00443	528.64	9.6
360		IN00444	468.54	8.9
361		IN00445	438.52	12
362		IN00447	384.43	15.2
363		IN00448	480.34	No inhibition

364		IN00449	480.34	3.4
365		IN00450	490.34	52.8
366		IN00452	445.89	23.2
367		IN00453	429.44	No inhibition
368		IN00454	456.45	No inhibition
369		IN00455	427.88	2.4

370		IN00456	162.14	6.2
371		IN00457	146.14	3.5
372		IN00458	162.14	4.7
373 [b]	 Scoparone	IN00459	206.19	6.7
374		IN00471	426.72	8.4
375		IN00475	246.22	70.8
376		IN00476	202.21	24.3
377		IN00477	254.24	10
378		IN00478	204.22	10.3
379		IN00480	288.04	4.4

380	 3'-Angeloyl-cis-khellactone	IN00482	344.36	3.3
381		IN00485	206.3	17.4
382		IN00488	298.30	31.5
383		IN00492	638.57	11.5
384		IN00495	600.61	30.6
385		IN00497	548.54	22.1
386		IN00498	360.31	31.3
387		IN00499	390.38	6.3
388		IN00500	374.34	33.3

389		IN00502	390.38	1.1
390		IN00503	246.30	4.8
391		IN00504	172.18	No inhibition
392		IN00505	172.18	8.89
393		IN00506	230.26	9.1
394		IN00507	263.2	No inhibition

[a]This repository consists of natural products, plus semi-synthetic derivatives along with few synthetic small molecules.

[b]IN0044 and IN00459 codes belong to the same compound 'scoparone'. These were submitted two times to the IIIM NP repository, therefore have two different codes. They differ from each other only in terms of their purity. IN0044 is 88% pure whereas IN00459 is 92% pure.

[c]IN00236 and IN00305 codes belong to the same compound 'coumurrayin'. These were submitted two times to the IIIM NP repository, therefore have two different codes. They differ from each other only in terms of their purity. IN00236 is 92% pure whereas IN00305 is 95% pure.

[d]IN00280 and IN00322 codes belong to the same compound 'betulin'. These were submitted two times to the IIIM NP repository, therefore have two different codes. They differ from each other only in terms of their purity. IN00280 is 94% pure whereas IN00322 is 98% pure.

[e]IN00281 and IN00324 codes belong to the same compound 'betulinic acid'. These were submitted two times to the IIIM NP repository, therefore have two different codes. They differ from each other only in terms of their purity. IN00280 is 92% pure whereas IN00322 is 96% pure.

S3. HPLC purity of In-house natural product library hits.

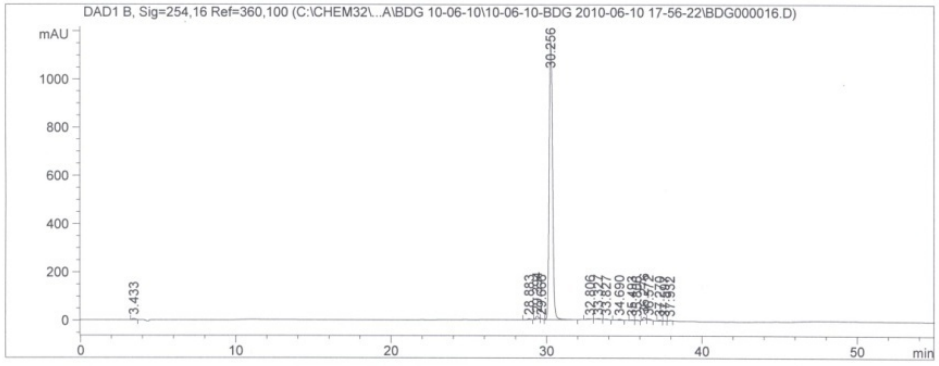
S3.1. Bergapten (IN-88) HPLC purity: 95.91%.

HPLC method:

Mobile Phase	1% Acetic acid in Water (A), Methanol (B), gradient Time 0.01 5 35 40 50 55 B (%) 10 10 100 100 10 10
Flow rate	0.7 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	55 min.
wavelength	254 nm.

Data File C:\CHEM32\1\DATA\BDG 10-06-10\10-06-10-BDG 2010-06-10 17-56-22\BDG000016.D
Sample Name: Bergapten

```
Acq. Operator   : R.K.GUPTA                      Seq. Line :  15
Acq. Instrument : Instrument 1                    Location : Vial 13
Injection Date  : 6/11/2010 8:43:55 AM                Inj :   1
                                                 Inj Volume : 10 µl
Acq. Method     : C:\Chem32\1\DATA\BDG 10-06-10\10-06-10-BDG 2010-06-10 17-56-22\RKG.M
Last changed    : 6/10/2010 5:55:20 PM by R.K.GUPTA
Analysis Method : C:\CHEM32\1\METHODS\S-3.M
Last changed    : 6/11/2010 1:00:28 PM by R.K.GUPTA
                  (modified after loading)
Sample Info     : Methanol (B),1.0% acetic acid in water (A), Column RP-1
                  8 (E-Merck, 5um,4.0 x 250 mm ), column temp. 30 degree,
                   Flow rate 0.7 ml/min.
                  Time     0.01      5.0     35     40     50     55
                  B(%)       10       10    100    100     10     10
```




```
                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.433 BB    0.2157   72.91894    5.39506   0.4067
   2  28.883 BB    0.2006   54.06482    4.18695   0.3015
   3  29.394 BB    0.1890  140.57851   12.33149   0.7840
   4  29.666 BB    0.1574   16.08510    1.68494   0.0897
   5  30.256 BB    0.2377 1.71978e4  1155.05298  95.9134
   6  32.806 BB    0.3462   43.86551    1.68214   0.2446
   7  33.327 BB    0.2754   50.43184    2.92155   0.2813
   8  33.827 BB    0.2255   27.36444    1.90579   0.1526
```

Data File C:\CHEM32\1\DATA\BDG 10-06-10\10-06-10-BDG 2010-06-10 17-56-22\BDG000016.D
Sample Name: Bergapten

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
9	34.690	BB	0.2547	65.64255	3.97375	0.3661
10	35.493	BB	0.1767	20.66164	1.93366	0.1152
11	35.806	BB	0.1710	21.60582	2.11805	0.1205
12	36.276	BB	0.1791	111.67329	10.41345	0.6228
13	36.572	BB	0.1959	40.02814	3.24607	0.2232
14	37.270	BB	0.1762	34.78351	3.26784	0.1940
15	37.597	BB	0.1651	12.62341	1.30183	0.0704
16	37.932	BB	0.1743	20.42071	1.98019	0.1139
Totals :				1.79305e4	1213.39574	

*** End of Report ***

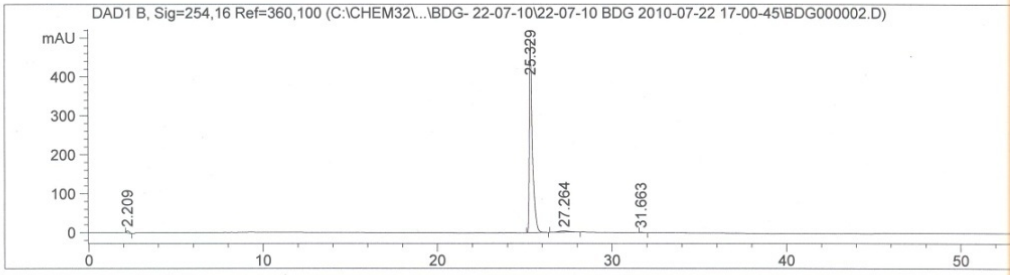
S3.2 Plumbagin (IN-114) HPLC purity: 96.55%.

HPLC Method:

Mobile Phase	Methanol(B), 1% Acetic acid in water Time 0.01 5 35 40 50 55 B (%) 10 10 100 100 10 10
Flow rate	0.7 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	55 min.
wavelength	254 nm.

Data File C:\CHEM32\1\DATA\BDG- 22-07-10\22-07-10 BDG 2010-07-22 17-00-45\BDG000002.D
Sample Name: Plumbagin

```
Acq. Operator   : R.K.GUPTA                      Seq. Line :   2
Acq. Instrument : Instrument 1                    Location : Vial 41
Injection Date  : 7/22/2010 6:29:09 PM                Inj :   1
                                                 Inj Volume : 10 µl
Acq. Method     : C:\Chem32\1\DATA\BDG- 22-07-10\22-07-10 BDG 2010-07-22 17-00-45\BDG.M
Last changed    : 7/22/2010 5:00:43 PM by R.K.GUPTA
Analysis Method : C:\CHEM32\1\DATA\SKK 9-12-09\9-12-09 SKK 2009-12-09 16-18-45\SKK000001.
                  D\DA.M (KKG.M)
Last changed    : 7/21/2010 10:09:50 AM by R.K.GUPTA
                  (modified after loading)
Sample Info     : Methonol (B) , 1.0%  Acetic Acid in Water ,Chromolith R
                  P-18e(Merck 100 -4.6 ), Column temp. 30 degree, Flow ra
                  te 0.7 ml/min.
                  Time: 0.01    5     35    40     50    55
                  B%     10     10  100   100     10     10
```




```
                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,16 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.209 BB    0.1788   57.26671    4.95758   0.9398
   2  25.329 BB    0.1675 5883.11719  499.06235  96.5513
   3  27.264 BB    0.5945  142.01895    3.10014   2.3308
   4  31.663 BB    0.1151   10.85110    1.33154   0.1781

Totals :                  6093.25395  508.45162


                          *** End of Report ***
```

S3.3. Karanjin (IN-195) HPLC purity : 90%

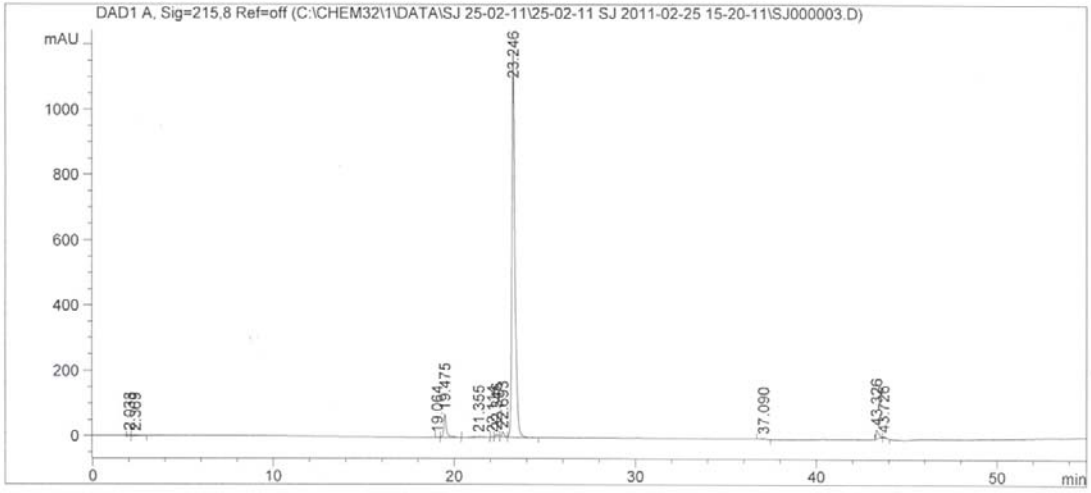
HPLC Method:

Mobile Phase	Methanol(B), 1% Acetic acid in water Time 0.01 5 35 40 50 55 B (%) 10 10 100 100 10 10
Flow rate	0.7 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	55 min.
wavelength	254 nm.

Data File C:\CHEM32\1\DATA\SJ 25-02-11\25-02-11 SJ 2011-02-25 15-20-11\SJ000003.D
Sample Name: Karanjin

Acq. Operator	: R.K.Gupta	Seq. Line	: 3
Acq. Instrument	: Instrument 1	Location	: Vial 72
Injection Date	: 2/25/2011 5:45:36 PM	Inj	: 1
		Inj Volume	: 5 µl
Acq. Method	: C:\Chem32\1\DATA\SJ 25-02-11\25-02-11 SJ 2011-02-25 15-20-11\KKG-2.M		
Last changed	: 2/25/2011 3:20:07 PM by R.K.Gupta		
Analysis Method	: C:\CHEM32\1\DATA\BS-AT\BS24-02-11 2011-02-24 16-30-40\PD000002.D\DA.M (BS-125GRAD.M)		
Last changed	: 2/28/2011 12:07:56 PM by Baljinder Singh (modified after loading)		
Sample Info	: Acetonitrile (B), Water (A), Column RP-18e (e- Merck, c romolith, 4.6 x 100 mm), Column temp. 30 degree, Flow rate 0.8 ml/min.		

Time	0.01	5	35	40	50	55
B(%)	10	10	100	100	10	10



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=215,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.038	BB	0.1498	61.96481	5.57096	0.4100
2	2.369	BB	0.2901	73.66037	3.18190	0.4874
3	19.064	BB	0.1115	9.98880	1.42938	0.0661
4	19.475	BB	0.1473	712.07123	70.80613	4.7115

Data File C:\CHEM32\1\DATA\SJ 25-02-11\25-02-11 SJ 2011-02-25 15-20-11\SJ000003.D
Sample Name: Karanjin

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
5	21.355	BB	0.5600	103.80831	2.27632	0.6869
6	22.114	BB	0.1037	7.07097	1.11869	0.0468
7	22.346	BB	0.1224	65.77806	8.48544	0.4352
8	22.693	BB	0.1332	125.49791	14.18733	0.8304
9	23.246	BB	0.1675	1.35961e4	1187.95898	89.9598
10	37.090	BB	0.3092	42.82722	2.07185	0.2834
11	43.326	BB	0.1434	294.11215	28.25551	1.9460
12	43.726	BB	0.1175	20.64273	2.57646	0.1366
Totals :				1.51135e4	1327.91897	

*** End of Report ***

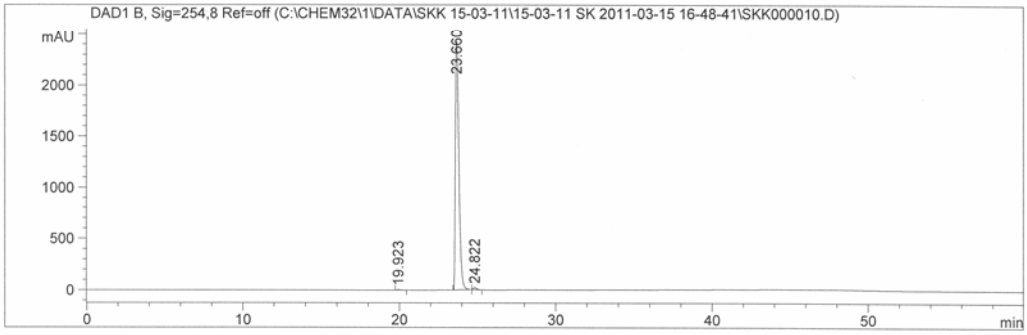
S3.4. Khellin (IN-199) HPLC purity : 99.05%

HPLC Method:

Mobile Phase	Methanol(B), 1% Acetic acid in water Time 0.01 5 35 40 50 55 B (%) 10 10 100 100 10 10
Flow rate	0.7 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	60 min.
wavelength	254 nm.

Data File C:\CHEM32\1\DATA\SKK 15-03-11\15-03-11 SK 2011-03-15 16-48-41\SKK000010.D
Sample Name: Khellin

```
Acq. Operator   : R.K.Gupta                      Seq. Line :  10
Acq. Instrument : Instrument 1                    Location : Vial 7
Injection Date  : 3/16/2011 2:17:58 AM                 Inj :  1
                                                 Inj Volume : 10 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 5 µl
Acq. Method     : C:\Chem32\1\DATA\SKK 15-03-11\15-03-11 SK 2011-03-15 16-48-41\KKG-2.M
Last changed    : 3/15/2011 4:47:05 PM by R.K.Gupta
Analysis Method : C:\CHEM32\1\METHODS\KKG-2.M
Last changed    : 3/16/2011 12:30:07 PM by R.K.Gupta
                  (modified after loading)
Sample Info     : Methanol(B):Water(A),Column RP-18(E-Merck,Chromolith,4.
                  6x100mm),Column temp. 30 c degree Flow rate 0.8mll/min
                  Time: 0.01      5      40       45      55     60
                  B(%):    10      10    100      100     10     10
```

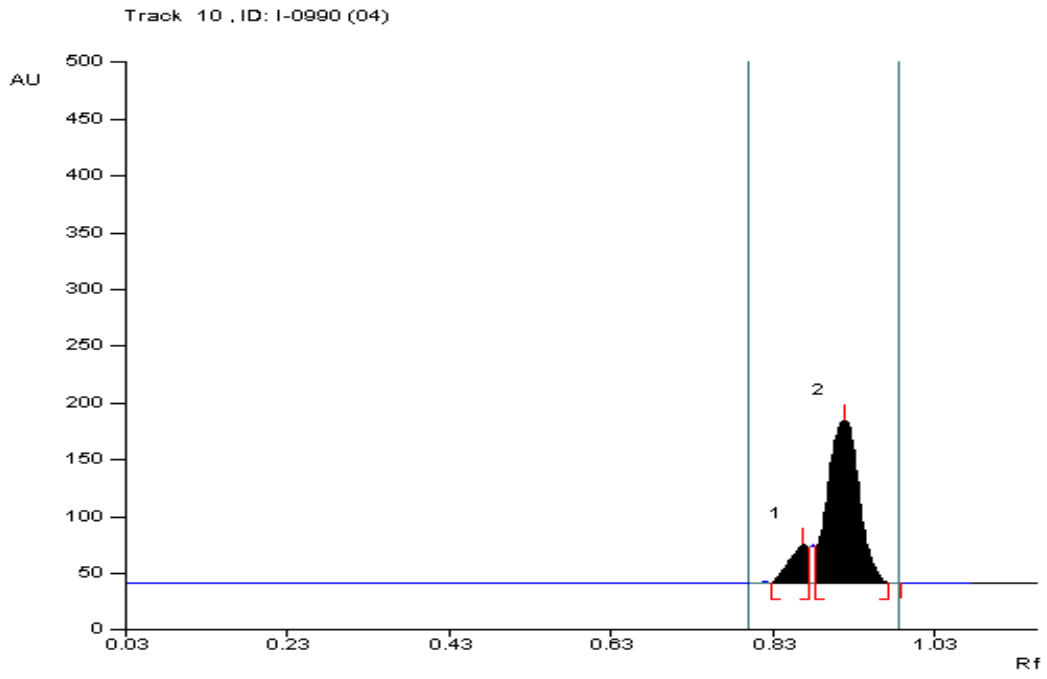



```
                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=254,8 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  19.923 BB    0.1696   59.10330    5.08640   0.1527
   2  23.660 BB    0.2443 3.83388e4  2427.37646  99.0595
   3  24.822 BB    0.2072  304.90768   22.34360   0.7878

Totals :                  3.87028e4  2454.80646


                          *** End of Report ***
```

S3.5. Anisomalin (IN-226) HPTLC % purity : 87.53%.

HPTLC Method:

Mobile Phase	**$CHCl_3$: Methanol (9:1)**
wavelength	500 nm.
Spray reagent	Cerric ammonium sulphate

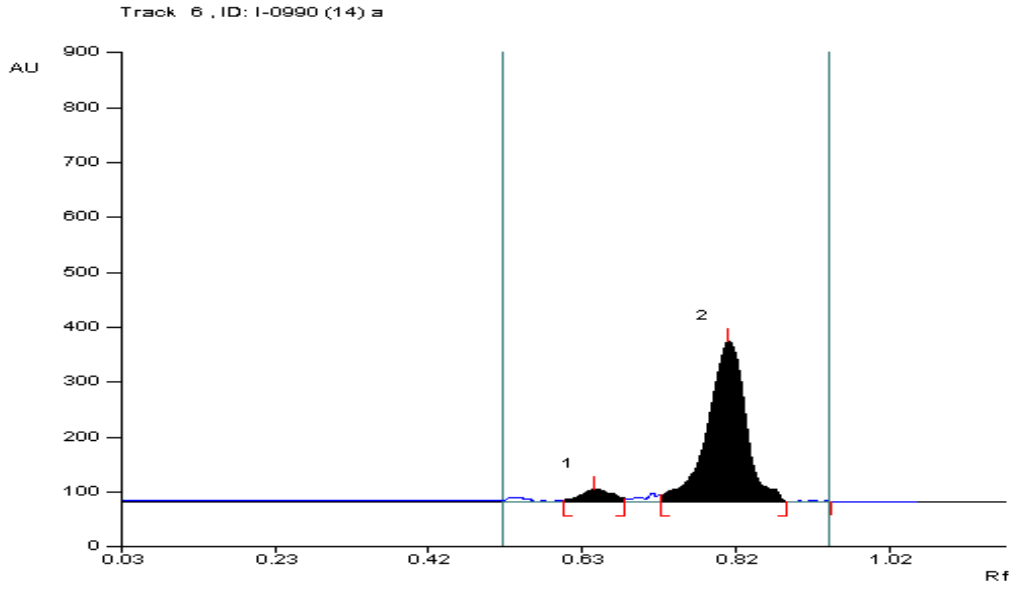


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assi
1	0.83 Rf	0.2 AU	0.87 Rf	35.1 AU	19.60 %	0.88 Rf	31.1 AU	563.2 AU	12.47 %	
2	0.88 Rf	33.1 AU	0.92 Rf	143.9 AU	80.40 %	0.97 Rf	0.3 AU	3952.1 AU	87.53 %	

S3.6. 6-methoxy dihydrochelerythrine (IN-229) HPTLC purity: 93.77%.

HPTLC Method:

Mobile Phase	**$CHCl_3$: Methanol (9:1)**
wavelength	500 nm.
Spray reagent	Dragendorff's reagent



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	As
1	0.60 Rf	2.4 AU	0.64 Rf	22.6 AU	7.17 %	0.68 Rf	6.2 AU	859.2 AU	6.23 %	
2	0.73 Rf	12.8 AU	0.82 Rf	292.9 AU	92.83 %	0.89 Rf	0.8 AU	12934.2 AU	93.77 %	

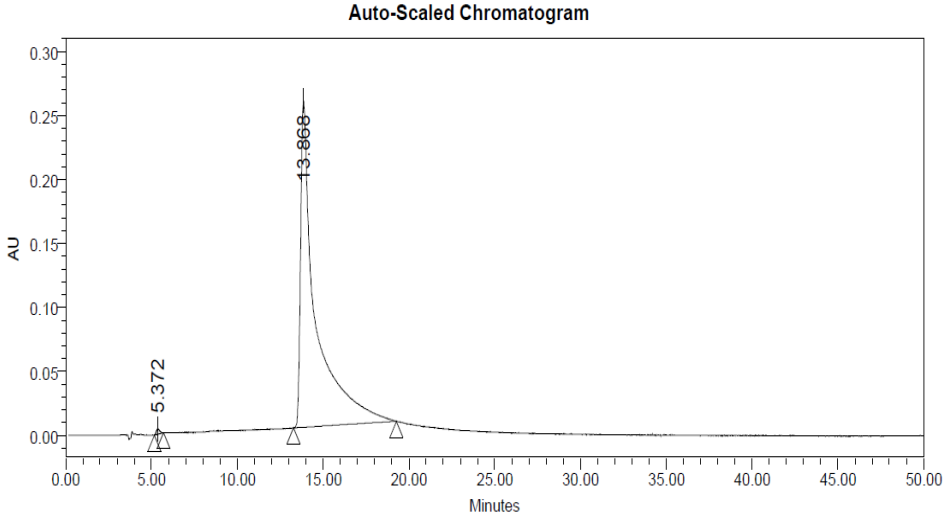
S3.7. Pongamol (IN-299) HPLC purity: 99%.

HPLC Method:

Mobile Phase	Methanol(B) : Water : Acetic acid (isocratic) 85 : 13.5 : 1.5
Flow rate	0.5 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	50 min.
wavelength	350 nm.

SAMPLE INFORMATION

Sample Name:	Pongamol	Acquired By:	System
		Date Acquired:	10/25/2011 4:41:34 PM
Sample Type:	Standard	Acq. Method Set:	Pongamol
		Date Processed:	10/27/2011 11:19:08 AM
Vial:	5	Processing Method:	pongamol
Injection #:	1	Channel Name:	W2996 350.0nm-1.2
Injection Volume:	10.00 ul	Proc. Chnl. Descr.:	W2996 PDA 350.0 nm at 1.2
Run Time:	50.0 Minutes	Flow rate: 0.5 ml/min	
Sample Set Name:	Pongamol	Sample conc:0.3 mg/mL MeOH	
column_name	RP-18,5um	Mobile phase: MeOH:H2O:AcOH(85:13.5:1.5)	



	RT	Area (µV*sec)	% Area	Height (µV)
1	5.372	59974	0.38	4217
2	13.868	15660961	99.62	255189

S3.8. Phellopterin (IN-309) HPC purity: 99%.

HPLC Method:

Mobile Phase	ACN (B), 1.5% Acetic acid in water (A) Time 0.01 5 65 67 70 75 B (%) 8 8 75 75 8 8
Flow rate	1 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	75 min.
wavelength	254 nm.

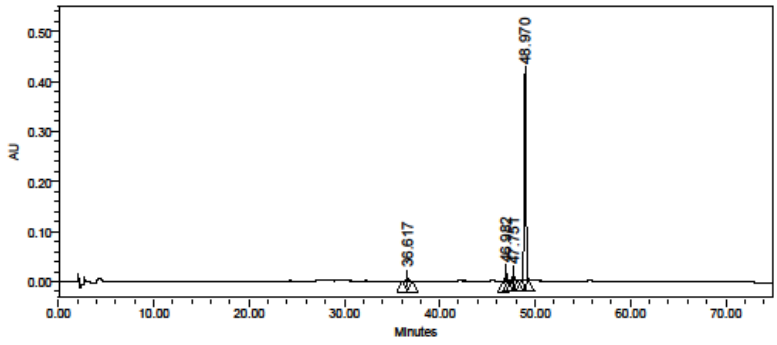
NPC DIVISION IIIM JAMMU

Reported by User: System Project Name: NPC8

SAMPLE INFORMATION

Sample Name:	Phellopterin	Acquired By:	System
Sample Type:	Standard	Date Acquired:	1/29/2012 2:37:26 AM
Vial:	10	Acq. Method Set:	Belerica 1
Injection #:	1	Date Processed:	1/30/2012 12:28:44 PM
Injection Volume:	10.00 ul	Processing Method:	Coumarins
Run Time:	75.0 Minutes	Channel Name:	W2996 254.0nm-1.2
Sample Set Name:	Coumarin	Proc. Chnl. Descr.:	W2996 PDA 254.0 nm at 1.2
column_name	RP-18,5um	Flow rate: 1 ml/min	
		Sample conc:0.5 mg/mL MeOH	
		Mobile phase: ACN:1.5%AcOH in water(gradient)	

Auto-Scaled Chromatogram



	RT	Area (µV*sec)	% Area	Height (µV)
1	36.617	54569	0.97	4610
2	46.982	157289	2.80	13557
3	47.751	165824	2.95	10385
4	48.970	5244668	93.28	411471

Report Method: Parveen Printed 12:13:16 PM 1/31/2012 Page: 1 of 1

S3.9. (IN-333) HPC purity: 99.5%.

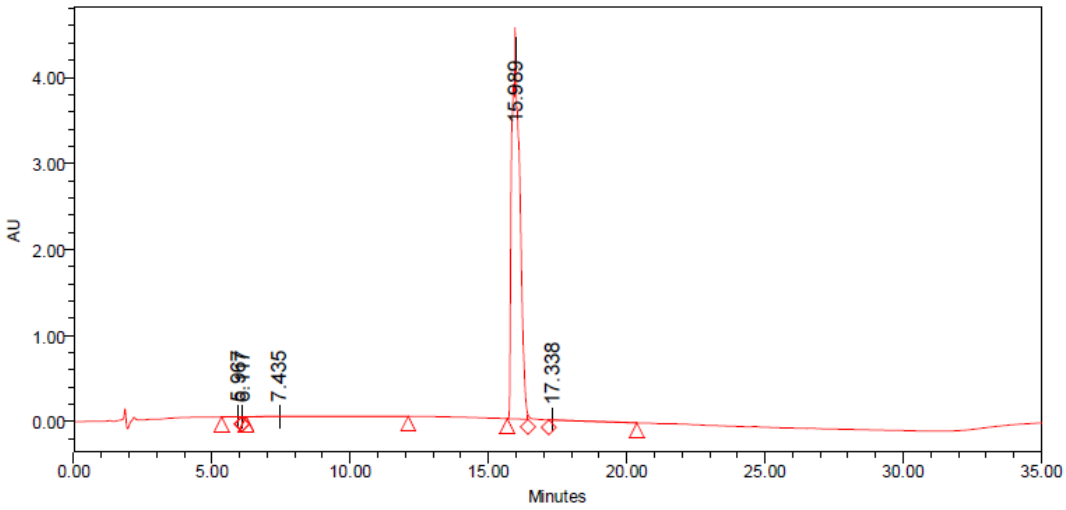
HPLC Method:

Mobile Phase	ACN (B), 1.5% Acetic acid in water (A) Time 0.01 5 20 25 30 35 B (%) 5 5 90 90 5 5
Flow rate	0.5 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	35 min.
wavelength	254 nm.

SAMPLE INFORMATION

Sample Name:	RM-47	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	REPOSITORY02052012
Vial:	2	Acq. Method Set:	Repository02052012
Injection #:	1	Processing Method:	reprository04052012
Injection Volume:	10.00 ul	Channel Name:	215.0nm
Run Time:	35.0 Minutes	Proc. Chnl. Descr.:	PDA 215.0 nm
Date Acquired:	5/2/2012 2:44:31 PM IST		
Date Processed:	5/7/2012 10:02:06 AM IST		

Auto-Scaled Chromatogram



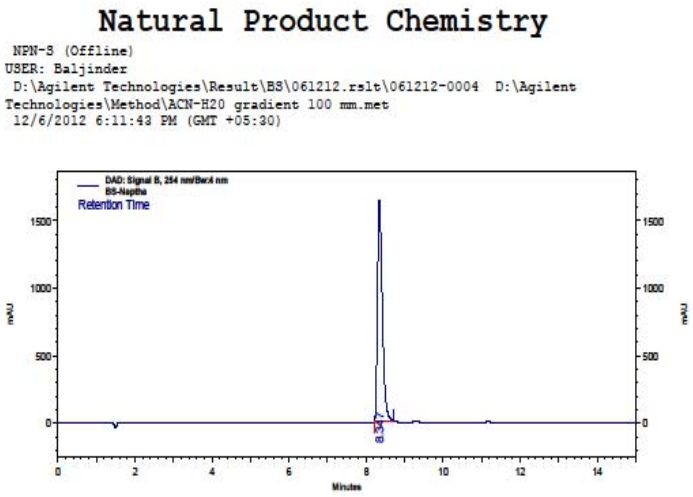
Peak Results

	Name	RT	Area	Height	% Area
1		5.967	3901	198	0.00
2		6.117	1009	189	0.00
3		7.435	222962	1393	0.25
4		15.989	89854010	4366314	99.55
5		17.338	180309	4241	0.20

S3.10. (IN-416) HPC purity: 99.99%.

HPLC Method:

Mobile Phase	ACN (B), water (A) gradient Time 0.0 10 15 18 B (%) 2 100 100 2
Flow rate	0.8 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	18 min.
wavelength	254 nm.



DAD: Signal B, 254 nm/Bw:4 nm
Results

Retention Time	Area Percent	Height Percent	Area	Height
8.347	100.000	100.000	29626002	3421722
Totals	100.000	100.000	29626002	3421722

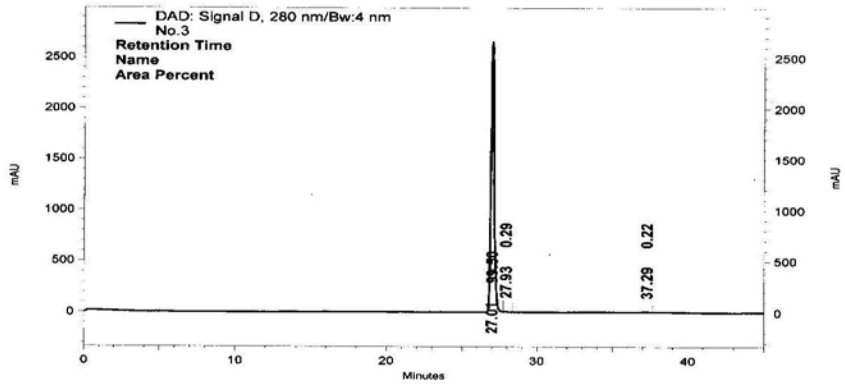
S3.11. (IN-475) HPLC purity: 99.50%.

HPLC Method:

Mobile Phase	ACN (B), water (A) gradient Time 0.0 30 35 40 45 B (%) 10 90 90 10 10
Flow rate	0.7 ml/min.
Column	Chromolith RP-18 (Merck, 5um, 4x250mm.)
Column Temp.	30 degree
Stop time	45 min.
wavelength	254 nm.

Area % Report

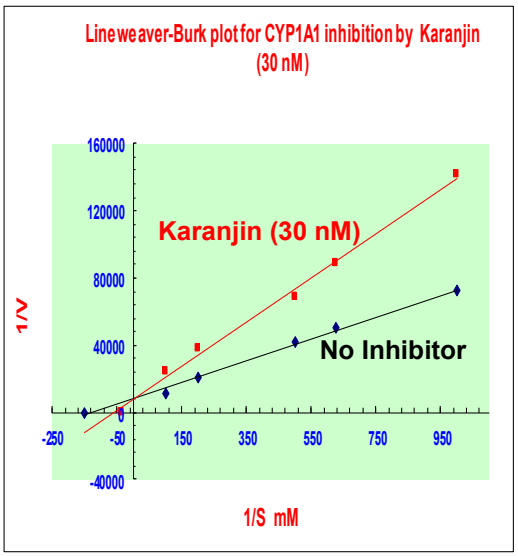
Data File: D:\Agilent Technologies\Result\SUNIL\repo 3,6,11,12,15,32,35,37,44.rslt\1
Method: D:\Agilent Technologies\Method\sunil\Sunil-GRAD 01.met
Acquired: 1/29/2013 10:40:21 PM (GMT +05:30)
Printed: 1/30/2013 10:54:56 AM (GMT +05:30)



DAD: Signal D, 280 nm/Bw:4 nm Results

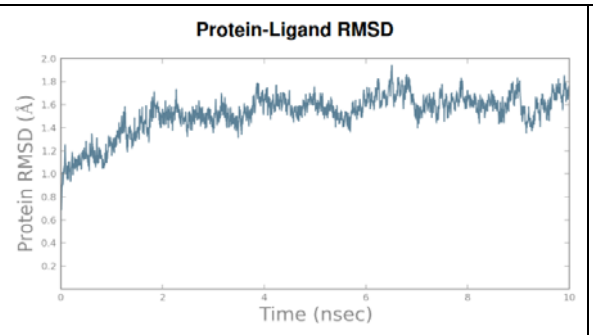
Name	Retention Time	Area	Height	Area %
	27.007	82999307	5567538	99.50
	27.927	240845	17516	0.29
	37.287	179449	8206	0.22
Totals		83419601	5593260	100.00

S4. Karanjin is a competitive inhibitor of CYP1A1

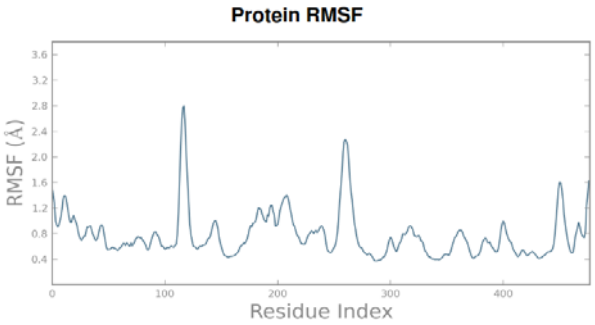


In case of Karanjin, Vmax is unaltered, while slope has changed while Km is increased from 6.6 mM to 23 mM. Karanjin was incubated with enzyme for 45 min. Similar results were obtained by incubation of karanjin with CYP1A1 for 5, 10, 20 and 30 min. This clearly shows that karanjin is a competitive inhibitor of CYP1A1.

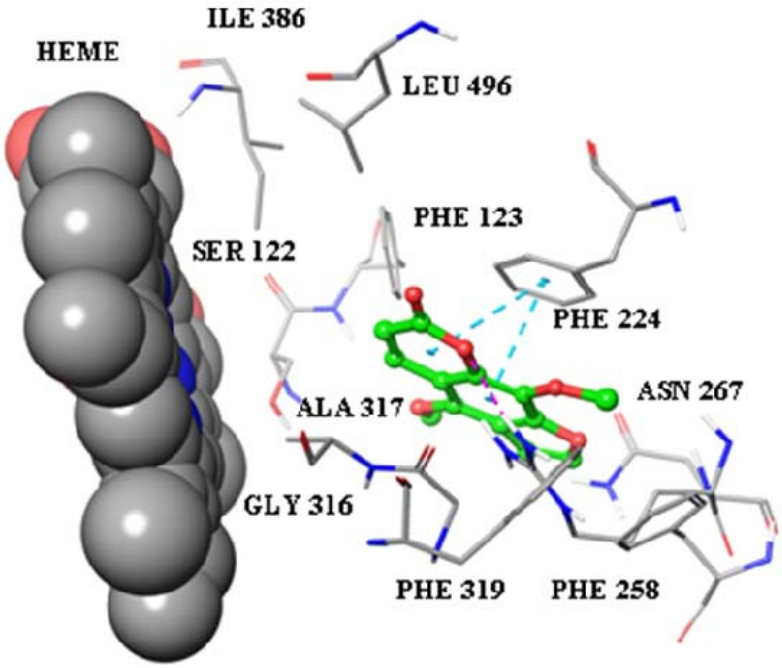
S5. MD simulation and interactions of isopimpinellin (**IN-475**) with CYP1A1



A

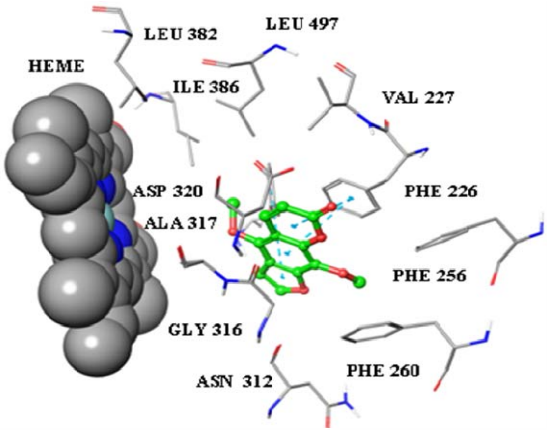


B

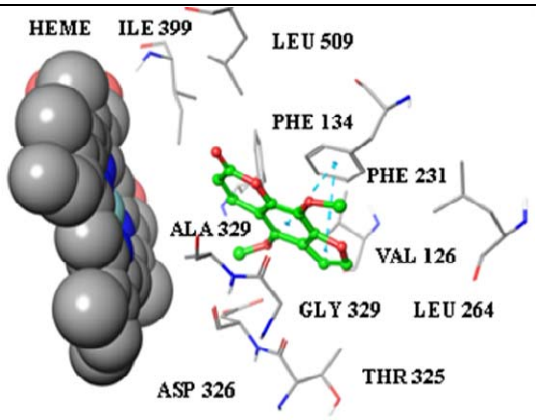


C

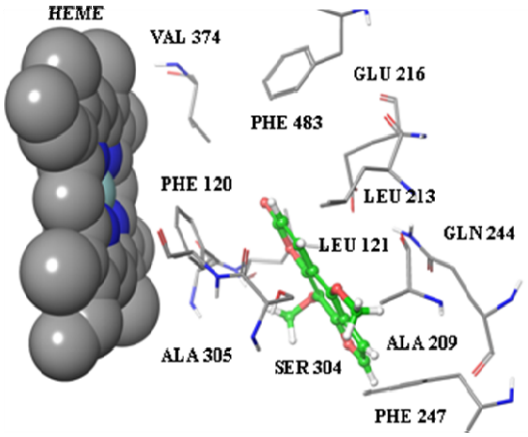
S6. Interactions of isopimpinellin (**IN-475**) with CYP1 CYP2 and CYP3 family isoform. A: CYP1A2, B: CYP1B1, C: CYP2D6, D: CYP3A4, E: CYP2C9. F: CYP2C19.



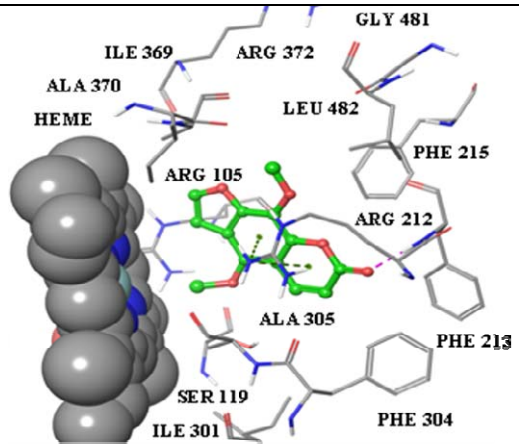
A



B



D



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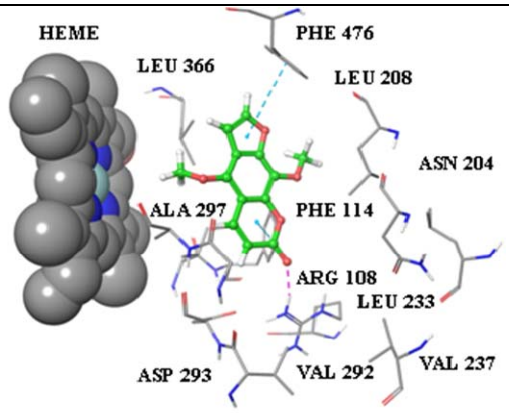

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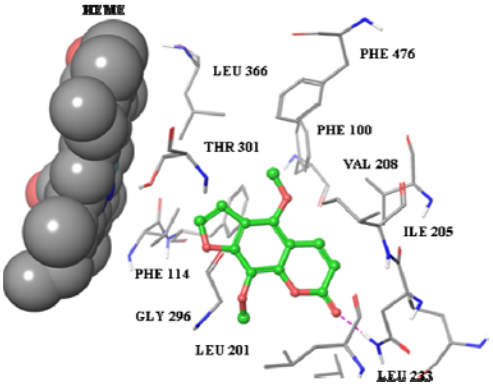
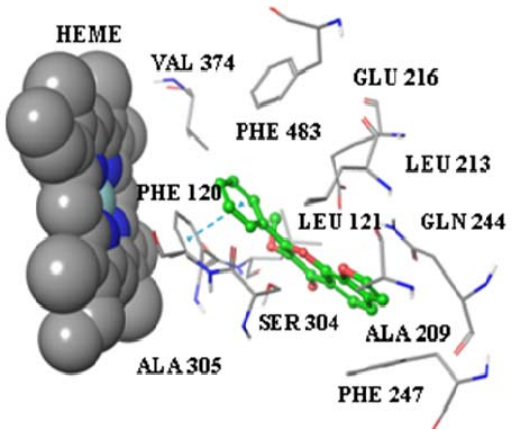
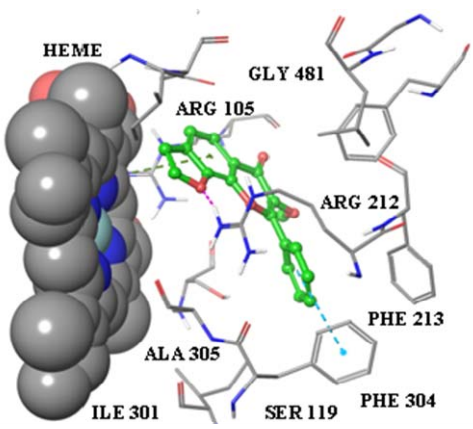


G

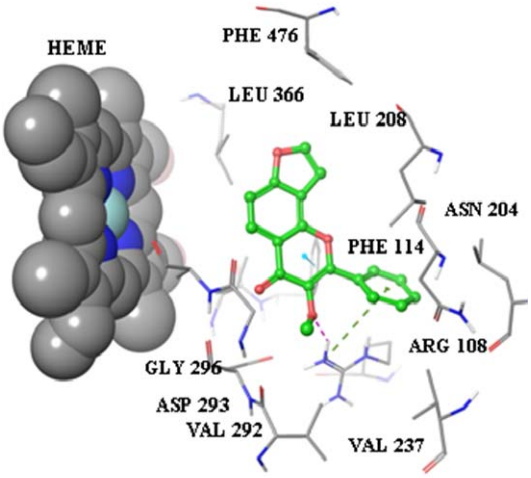
S7. Interactions of karanjin (**IN-195**) with CYP2 and CYP3 family isoform. A: CYP2D6, B: CYP3A4, C: CYP2C9. D: CYP2C19.



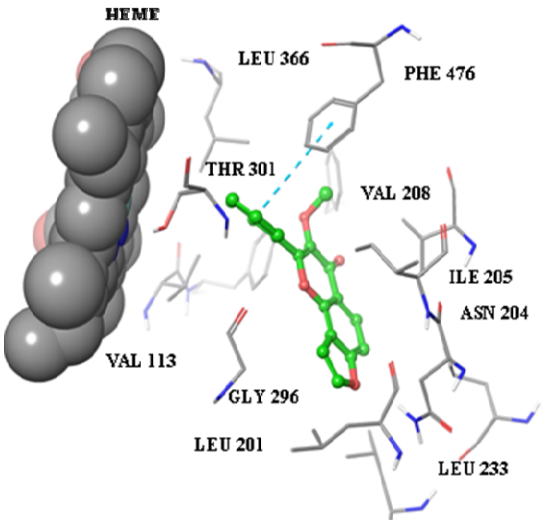
A



B



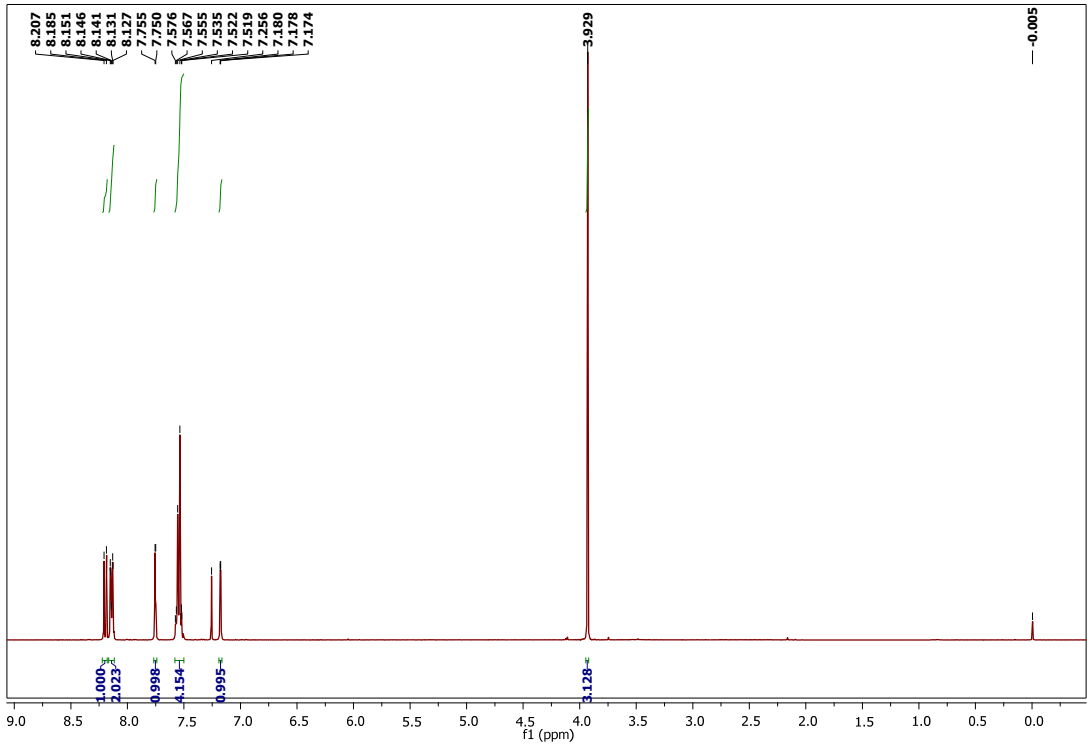
C

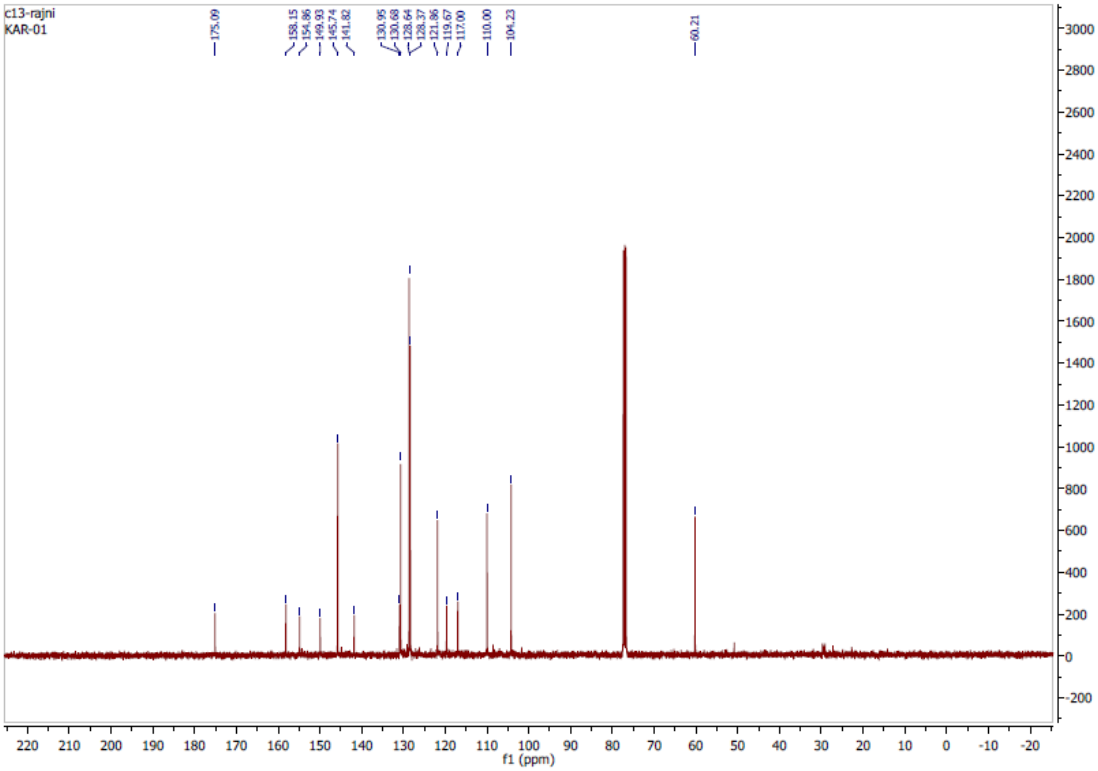


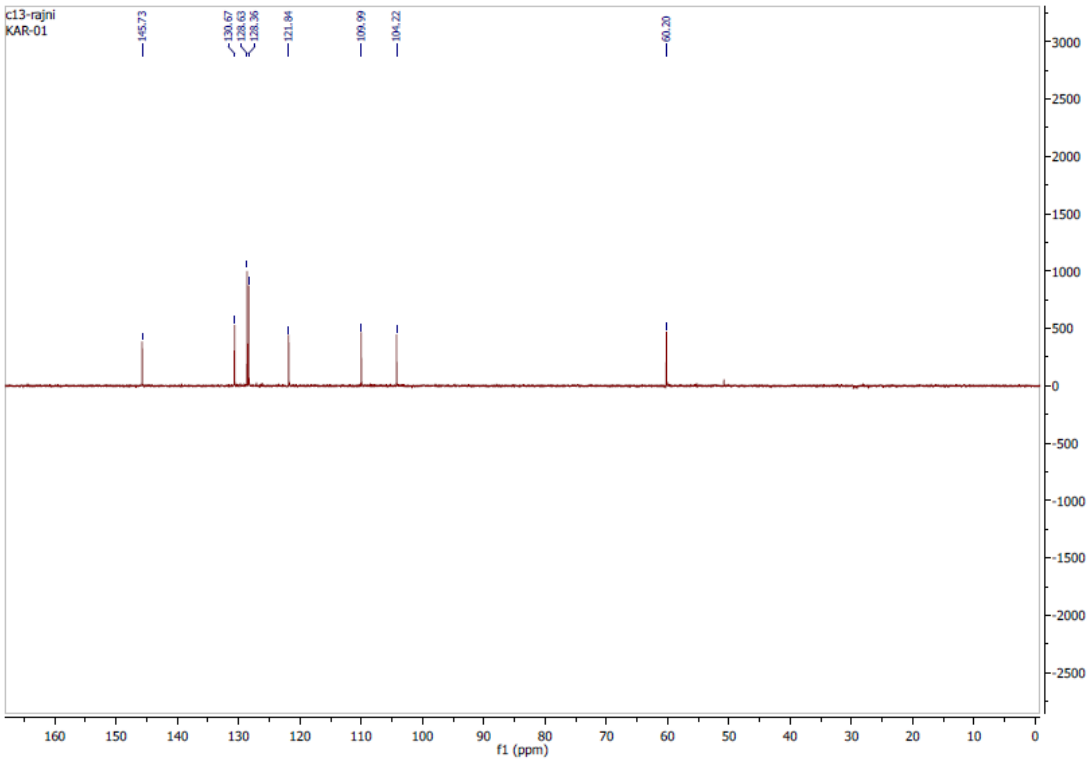
D

S8: NMR, HPLC and HRMS data scans of IN-195 and IN-475

S8.1. ^{1}H, ^{13}C NMR, DEPT135 NMR ($CDCl_3$) and HPLC of karanjin (**IN-195**)







HPLC method:

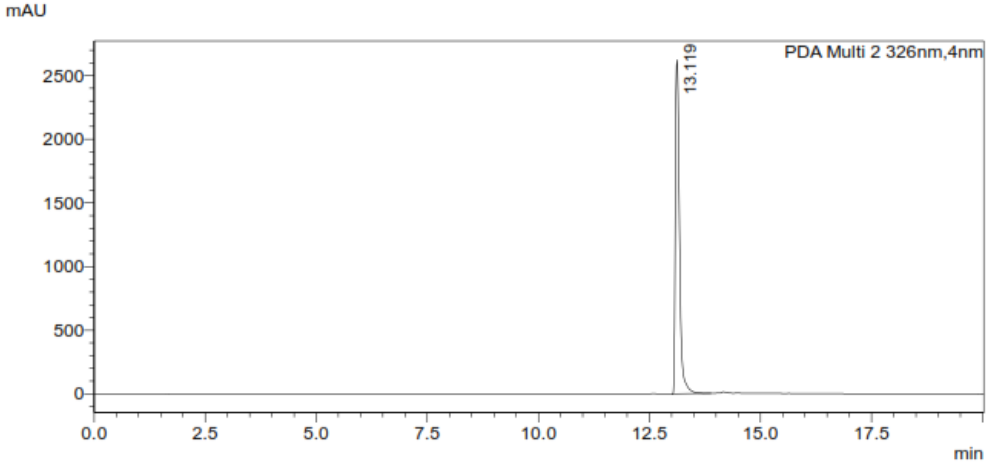
Compound	Mobile phase	Detection wavelength (nm)	Retention time (min)	% purity
IN-195	0.1% Formic acid- MeOH @ 1ml/min Injection volume 3uL Column oven temp. 37 °C Column C18 Chromolith® performance RP-18e (100-4.6 mm) 0.01 Pumps Pump B Conc. 70 2 Pumps Pump B Conc. 70 10 Pumps Pump B Conc. 10 12 Pumps Pump B Conc. 10 17 Pumps Pump B Conc. 70 20 Pumps Pump B Conc. 70 20.01 Controller Stop	326	13.119	100

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: IN-195		
Sample ID	:		
Data Filename	: IN-195.lcd		
Method Filename	: NPC-290 salt screening method file.lcm		
Batch Filename	: Surfactant screening- dissolution test-28th Sept 2017.lcb		
Vial #	: 1-51	Sample Type	: Unknown
Injection Volume	: 3 uL		
Date Acquired	: 29-09-2017 14:59:02	Acquired by	: System Administrator
Date Processed	: 29-09-2017 15:21:14	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

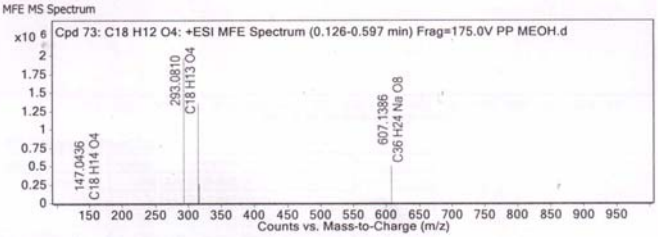
PDA Ch2 326nm

Peak#	Name	Ret. Time	Area%
1	IN-195	13.119	100.000
Total			100.000

The newly isolated 'karanjin' as a powdered form was later used to determine IC_{50} / Ki values and further cell based experiments. The HPLC purity of this sample is provided above.

Compound Label	m/z	RT	Algorithm	Mass
Cpd 73: C18 H12 O4	293.081	0.195	Find by Molecular Feature	292.0738

MFE MS Spectrum



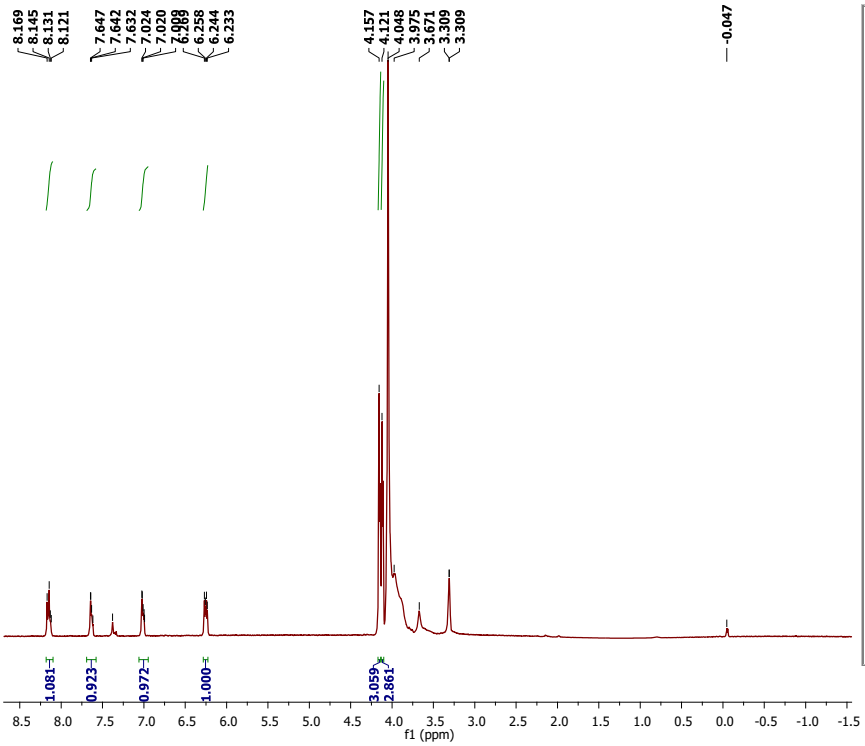
MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
147.0436	2	5580.82	C18 H14 O4	(M+2H)+2
293.081	1	1965383.38	C18 H13 O4	(M+H)+
294.0847	1	343819.56	C18 H13 O4	(M+H)+
315.0631	1	1340063.13	C18 H12 Na O4	(M+Na)+
316.0666	1	251879.34	C18 H12 Na O4	(M+Na)+
602.1804	1	1506.57	C36 H28 N O8	(2M+NH4)+
607.1386	1	496920.09	C36 H24 Na O8	(2M+Na)+
608.1413	1	180297.22	C36 H24 Na O8	(2M+Na)+

Predicted Isotope Match Table

Isotope	m/z	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
1	147.0436	147.0441	3.34	100	100	100	100

S8.2. ^{1}H NMR ($CDCl_3$ + CD_3OD), HPLC and HRESI-MS of isopimpinellin (**IN-475**)



	Parameter	Value
1	Data File Name	D:/ PJ analytical data/ prashant-NMR data 1H and 13 C/ NMR FID with PDF/ Natural products/ IN-475 1H NMR/ fid
2	Title	Natural products
3	Comment	IN-475
4	Origin	Bruker BioSpin GmbH
5	Owner	Administrator
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	MeOD
10	Temperature	300.0
11	Pulse Sequence	zgpr
12	Number of Scans	16
13	Receiver Gain	144
14	Relaxation Delay	0.5000
15	Pulse Width	13.5000
16	Acquisition Time	2.7263
17	Acquisition Date	2017-09-21T10:49:06
18	Modification Date	2017-09-21T18:57:20
19	Spectrometer Frequency	400.13
20	Spectral Width	12019.2
21	Lowest Frequency	-3105.1
22	Nucleus	1H
23	Acquired Size	32768
24	Spectral Size	65536

Compound	Mobile phase	Detection wavelength (nm)	Retention time (min)	% purity
IN-475	0.1% Formic acid- MeOH @ 1ml/min Injection volume 3uL Column oven temp. 37 °C Column C18 Chromolith® performance RP-18e (100-4.6 mm) 0.01 Pumps Pump B Conc. 70 2 Pumps Pump B Conc. 70 10 Pumps Pump B Conc. 10 12 Pumps Pump B Conc. 10 17 Pumps Pump B Conc. 70 20 Pumps Pump B Conc. 70 20.01 Controller Stop	267	10.289	99.774

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: IN-475		
Sample ID	:		
Data Filename	: IN-475.lcd		
Method Filename	: NPC-290 salt screening method file.lcm		
Batch Filename	: Surfactant screening- dissolution test-28th Sept 2017.lcb		
Vial #	: 1-52	Sample Type	: Unknown
Injection Volume	: 3 uL		
Date Acquired	: 29-09-2017 15:19:33	Acquired by	: System Administrator
Date Processed	: 29-09-2017 15:43:27	Processed by	: System Administrator

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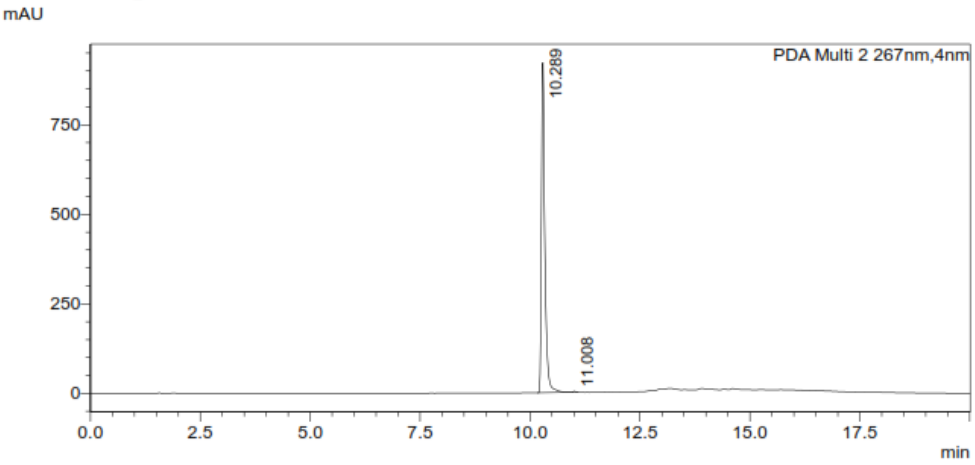


<Peak Table>

PDA Ch2 267nm

Peak#	Ret. Time	Area%
1	10.289	99.774
2	11.008	0.226
Total		100.000

Qualitative Compound Report

Data File	IN-475.d	**Sample Name**	IN-475
Sample Type	Sample	**Position**	Vial 12
Instrument Name	Instrument 1	**User Name**	
Acq Method	vishal_12-01-13.m	**Acquired Time**	27-09-2017 PM 3:24:32
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			

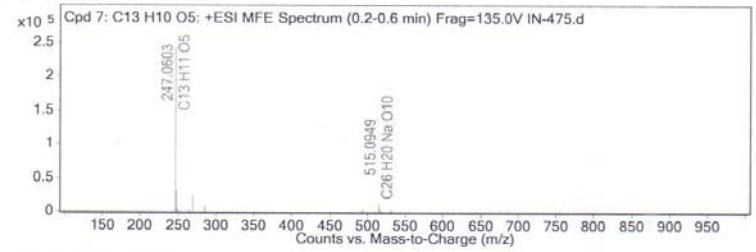
Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125)

Compound Table

Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB Formula
Cpd 7: C13 H10 O5	0.3	246.053	C13 H10 O5	C13 H10 O5	-0.81	C13 H10 O5

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 7: C13 H10 O5	247.0603	0.3	Find by Molecular Feature	246.053

MFE MS Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
247.0603	1	242862.34	C13 H11 O5	(M+H)+
248.0635	1	32038.57	C13 H11 O5	(M+H)+
249.0657	1	4479.71	C13 H11 O5	(M+H)+
269.0422	1	26075.36	C13 H10 Na O5	(M+Na)+
270.046	1	4631.81	C13 H10 Na O5	(M+Na)+
285.0159	1	9241.26	C13 H10 K O5	(M+K)+
493.1118	1	3327.8	C26 H21 O10	(2M+H)+
515.0949	1	12328.17	C26 H20 Na O10	(2M+Na)+
516.0976	1	3309.22	C26 H20 Na O10	(2M+Na)+
531.0688	1	2962.24	C26 H20 K O10	(2M+K)+

Predicted Isotope Match Table

Isotope	*m/z*	Calc m/z	Diff (ppm)	Abund %	Calc Abund %	Abund Sum %	Calc Abund Sum %
1	247.0603	247.0601	-0.92	100	100	86.71	85.8
2	248.0635	248.0635	0.01	13.19	14.38	11.44	12.34
3	249.0657	249.0656	-0.49	1.84	1.98	1.6	1.7
4	250.0685	250.0683	-0.91	0.3	0.19	0.26	0.16

--- End Of Report ---