Supporting Information

Synthesis and evaluation of the multi-target-directed ligands against Alzheimer's disease based on the fusion of donepezil and ebselen

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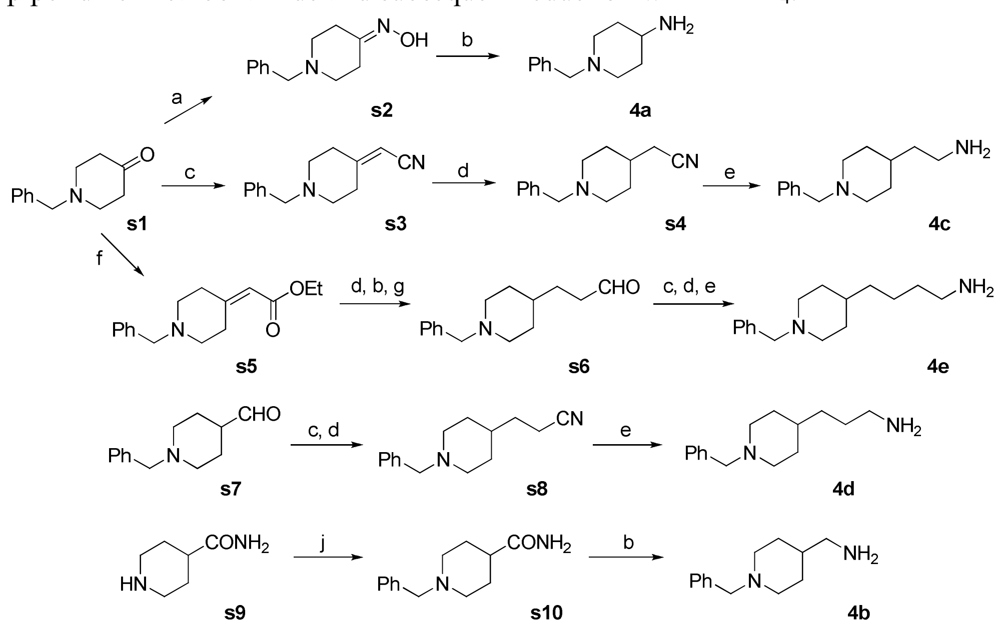
Contents

1. Experimental procedures for the synthesis of amine intermediates amine(4a-4e). First, the reaction of 1-benzylpiperidin-4-one **s1** with hydroxylamine hydrochloride, in the presence of potassium carbonate, produced oxime **s2**, which was reduced with $LiAlH_4$ to produce amine **4a**. Separately, **s1** reacted with diethyl cyanomethylphosphonate in the presence of K_2CO_3 to produce unsaturated nitrile **s3**. The hydrogenation of compound **s3** catalysed by Pt/C yielded nitrile **s4,** which was reduced with $LiAlH_4$ in THF at 0°C to produce amine **4c**. The synthesis of amine **4e** was similar to that of amine **4c**. The Wittig reaction of **s1** with triethyl phosphonoacetate produced unsaturated ester **s5**, which underwent hydrogenation, reduction by $LiAlH_4$ and oxidation to make aldehyde **s6,** successively. Following the same procedure used to prepare amine **4c**, compound **s6** was converted to amine **4e,** which has four carbon spacers between the piperidine ring and the amino group. Using 1-benzylpiperidine-4-carbaldehyde as the starting material, amine **4d** was obtained by the above method. Amine **4b** was obtained by benzylation of piperidine-4-carboxamide and subsequent reduction with $LiAlH_4$.



Scheme S1. Synthesis of amine intermediates **4a-4e**. Reagents and conditions: (a) Hydroxylamine hydrochloride, K_2CO_3, EtOH; (b) $LiAlH_4$, THF, reflux; (c) Diethyl cyanomethylphosphonate, K_2CO_3, THF; (d) Pt/C, H_2; (e) $LiAlH_4$, THF, 0℃; (f) Triethyl phosphonoacetate, K_2CO_3, THF; (g) Oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, -78℃; (j) BnBr, $NaHCO_3$, toluene.

1-benzylpiperidin-4-one oxime (s2). [1] 1-Benzyl-4-piperidone (4 g, 21.2 mmol) in dry EtOH (4 mL) was added to a mixture of hydroxylamine hydrochloride (2.95 g, 42.4 mmol) and K_2CO_3 (5.85 g, 42.4 mmol) in EtOH (16 mL). The reaction mixture was refluxed for 1 h and was filtered after cooling. The residue was washed with EtOH, and the filtrate was evaporated to yield oxime (**s2**) (3.81 g, 88%) as a solid, which was used without further purification.

4-benzylcyclohexanamine (4a). 1-Benzylpiperidin-4-one oxime (**s2**) (2.04 g, 10 mmol) in dry THF (5 mL) was added to a suspension of $LiAlH_4$ (1.0 g, 26.3 mmol) in dry THF (20 mL) at 0 °C, and the mixture was refluxed for 16 h. After cooling to room temperature, 1.0 mL of water was added slowly, and the solution was stirred for 15 min at 0 °C. One millilitre of 15% NaOH was added, the solution was stirred for another 15 min and 3.0 mL water was added. The mixture was filtered and washed with EtOAc and H_2O. The combined organic layers were dried over Na_2SO_4 and concentrated, resulting in a colourless oil (**4a**) (1.42 g, 75%) that was used without further purification.

1-benzylpiperidine-4-carboxamide (s10). [2] Benzyl bromide (13.0 mL, 110 mmol) was added slowly to a mixture of commercial piperidine-4-carboxamide (**s9**) (12.8 g, 100 mmol), $NaHCO_3$ (15.12 g, 180 mmol) and toluene (200 mL). The reaction mixture was refluxed for 2 h and filtered after cooling. The residue was dissolved in MeOH and filtered, and the filtrate was evaporated to yield the crude product. The brown solid was crystallised from acetone and MeOH to produce pure product (**s10**) (16.3 g, 75%).

(1-benzylpiperidin-4-yl)methanamine (4b) 1-Benzylpiperidine-4-carboxamide (**s10**) (3.27 g, 15 mmol) in dry THF (10 mL) was added to a suspension of $LiAlH_4$ (1.0 g, 26.3 mmol) in dry THF at 0 °C. The mixture was refluxed for 5 h. In a procedure similar to the production of compound **4a**, compound **4b** was produced as a colourless oil (0.624 g, 67%) that was used without further purification.

General procedure for the synthesis of amine (4c, 4d, 4e) from an aldehyde or ketone. [2]

(1) A mixture of K_2CO_3 (1 eq) and diethyl cyanomethylphosphonate (1.2 eq) in dry THF was stirred at room temperature for 15 min and refluxed for 20 min. After cooling, the aldehyde or ketone (1 eq) was added, and the mixture was refluxed for 12 h. After cooling, a 10% K_2CO_3 solution was added, and the mixture was extracted with EtOAc. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography, and a white solid was obtained.

(2) To a solution of the above product in MeOH, 10% Pt/C was added, filling the hydrogen to 300 psi. The reaction was stirred at room temperature for 10 h. The solution was then filtered, and the filtrate was concentrated to produce a colourless oil, which was used without further purification.

(3) The product from step 2 in dry THF was added to a suspension of $LiAlH_4$ at 0 °C, and the mixture was stirred at 0 °C for 1 h. In a process similar to the production of compound **3**, the amines were obtained and used without further purification.

2-(1-benzylpiperidin-4-yl)ethanamine (4c) Commercial 1-benzyl-4-piperidone was used as the starting reactant to make oil 2-(1-benzylpiperidin-4-yl) ethanamine (**4c**), yield: 61%.

3-(1-benzylpiperidin-4-yl)propan-1-amine (4d) Commercial 1-benzyl-4-formylpiperidine was used as the starting reactant to make 3-(1-benzylpiperidin-4-yl)propan-1-amine,yield: 64%.

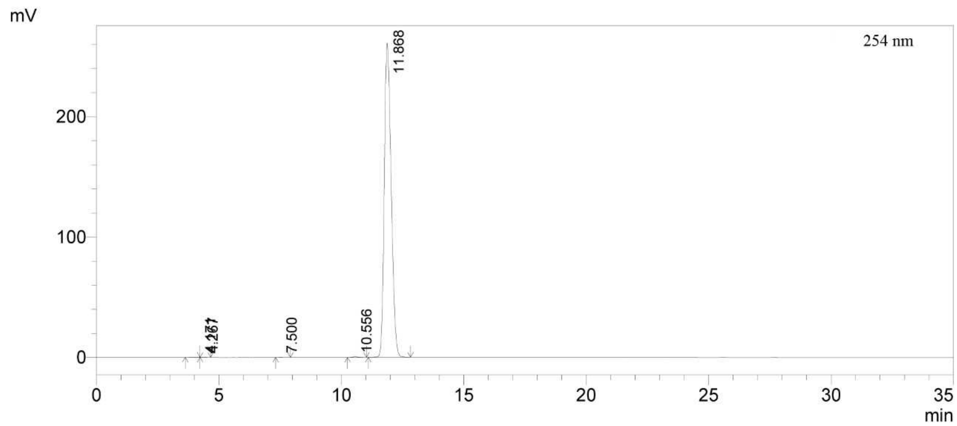
4-(1-benzylpiperidin-4-yl)butan-1-amine (4e) 2-(1-Benzylpiperidin-4-yl) acetaldehyde [3] was used as the starting reactant to make

4-(1-benzylpiperidin-4-yl)butan-1-amine, yield: 56%.

2. HPLC and FT-IR spectrum of the target compounds

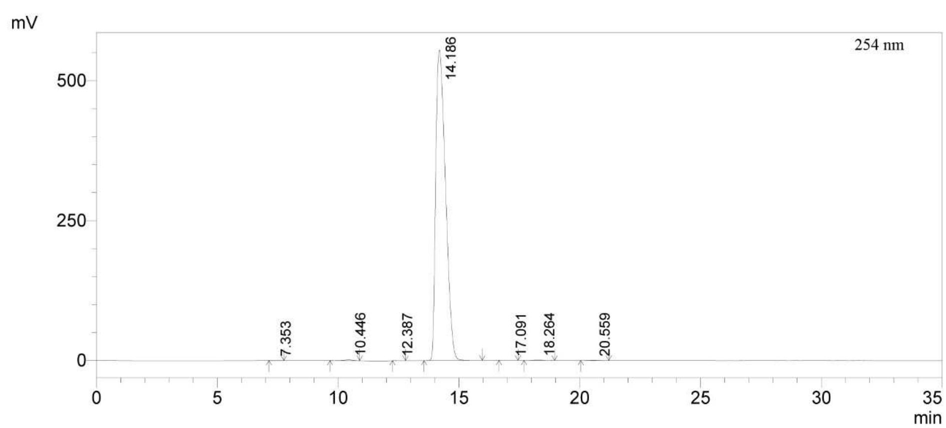
2.1 HPLC spectrum

5a



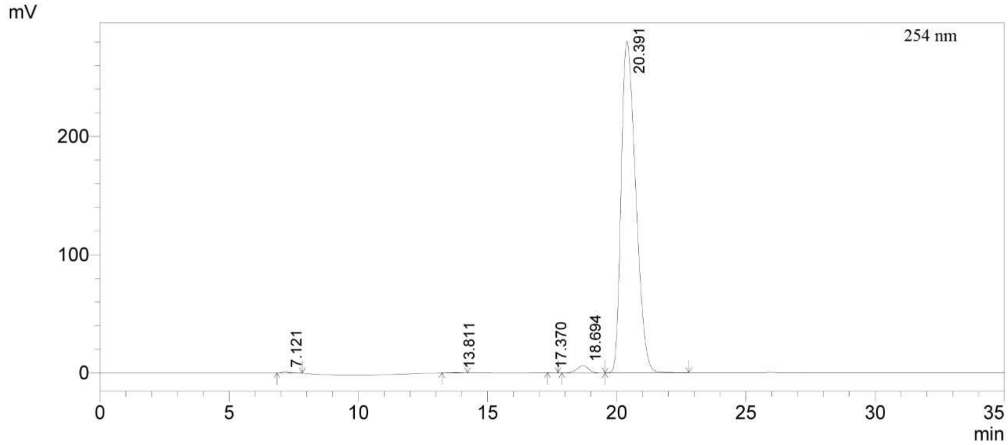
peak#	retention time	area	area %
1	4.171	4533	0.088
2	4.267	2666	0.052
3	7.500	2055	0.040
4	10.556	8260	0.161
5	11.868	5119801	99.659
total		5137314	100.000

5b



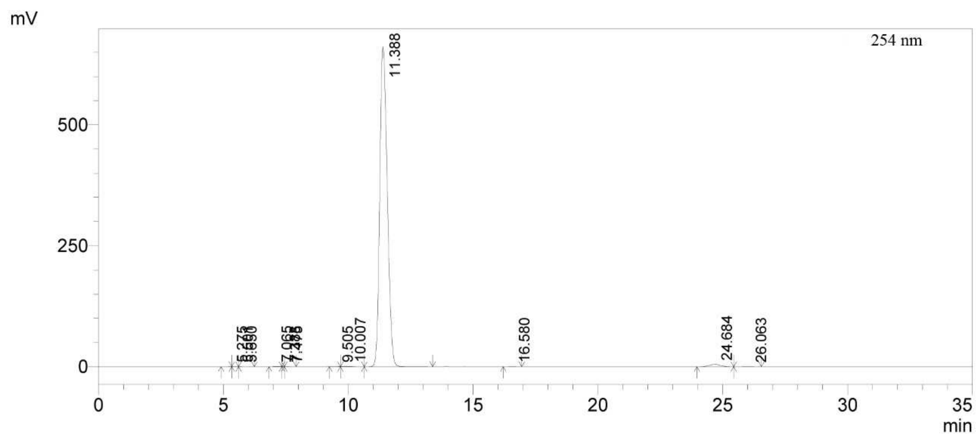
peak#	retention time	area	area %
1	7.353	2527	0.016
2	10.446	28632	0.183
3	12.387	1552	0.010
4	14.186	15549616	99.582
5	17.091	3438	0.022
6	18.264	16570	0.106
7	20.559	12546	0.080
total		15614881	100.000

5c



peak#	retention time	area	area %
1	7. 121	16464	0. 147
2	13. 811	7391	0. 066
3	17. 370	692	0. 006
4	18. 694	180496	1. 615
5	20. 391	10971566	98. 165
total		11176609	100. 000

5d



peak#	retention time	area	area %
1	5. 275	2965	0. 021
2	5. 501	2923	0. 021
3	5. 650	3080	0. 022
4	7. 065	9638	0. 069
5	7. 388	1182	0. 008
6	7. 475	2189	0. 016
7	9. 505	3959	0. 028
8	10. 007	13544	0. 097
9	11. 388	13814367	98. 613
10	16. 580	4650	0. 033
11	24. 684	143888	1. 027
12	26. 063	6215	0. 044
total		14008600	100. 000

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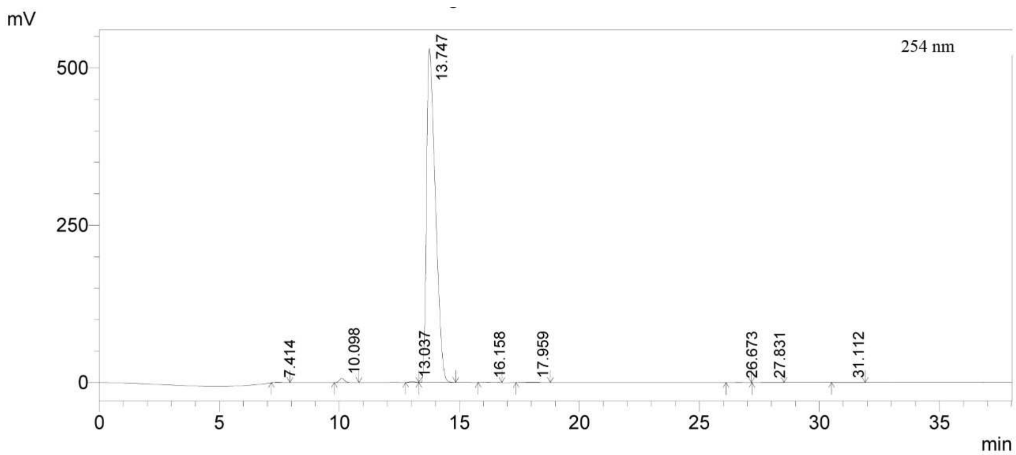
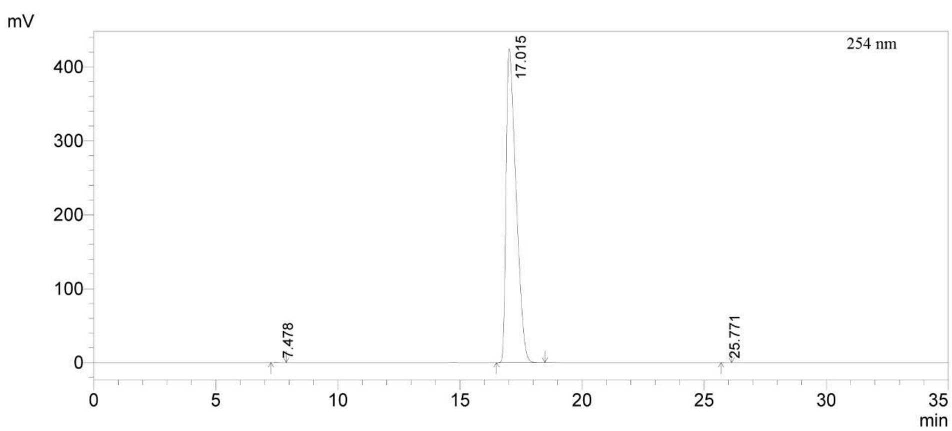


peak#	retention time	area	area %
1	7.414	8042	0.058
2	10.098	94358	0.686
3	13.037	19470	0.142
4	13.747	13597137	98.904
5	16.158	4952	0.036
6	17.959	8191	0.060
7	26.673	2953	0.021
8	27.831	4372	0.032
9	31.112	8358	0.061
total		13747833	100.000

6b



peak#	retention time	area	area %
1	7.478	2446	0.020
2	17.015	11983005	99.965
3	25.771	1730	0.014
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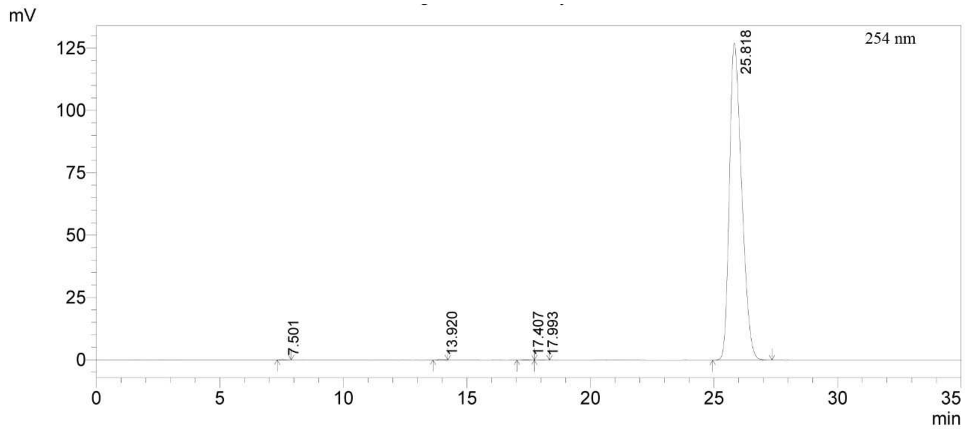

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1	7.501	2271	0.052
2	13.920	3006	0.068
3	17.407	3225	0.073
4	17.993	2294	0.052
5	25.818	4396982	99.755
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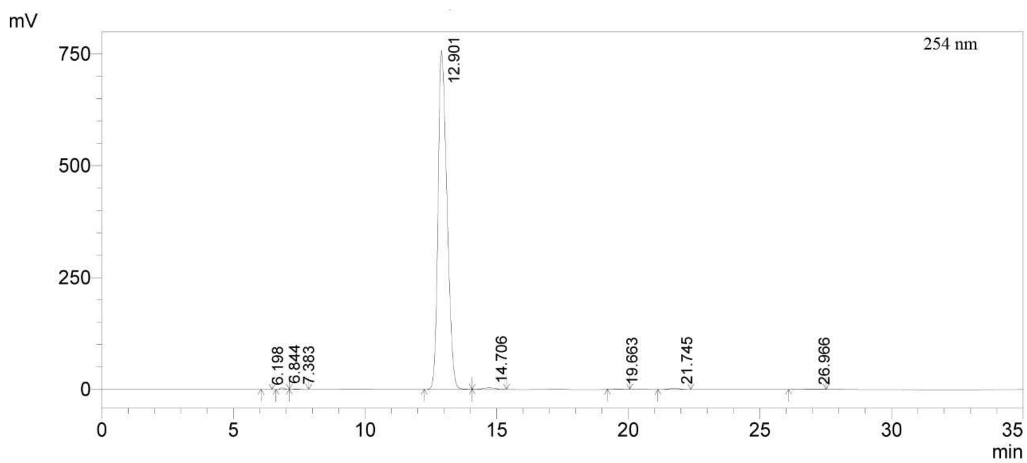

peak#	retention time	area	area %
1	6.198	1462	0.008
2	6.844	33855	0.187
3	7.383	12794	0.071
4	12.901	17904688	98.761
5	14.706	103394	0.570
6	19.663	8614	0.048
7	21.745	48330	0.267
8	26.966	16169	0.089
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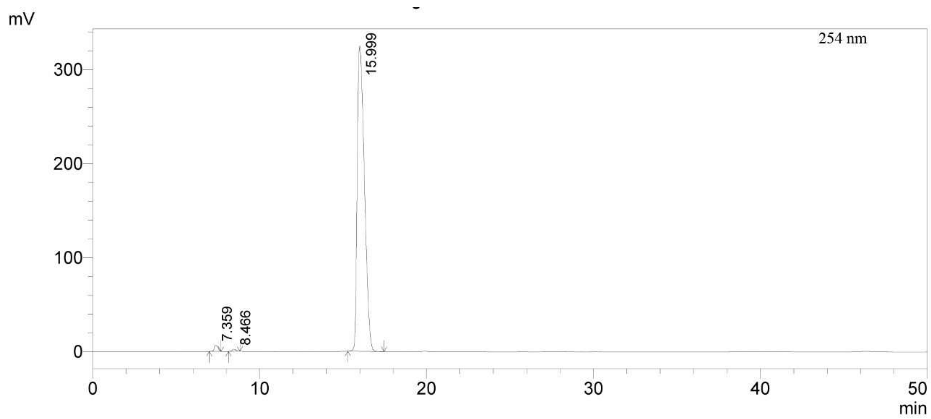

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1	7. 359	101580	1. 025
2	8. 466	35899	0. 362
3	15. 999	9776487	98. 613
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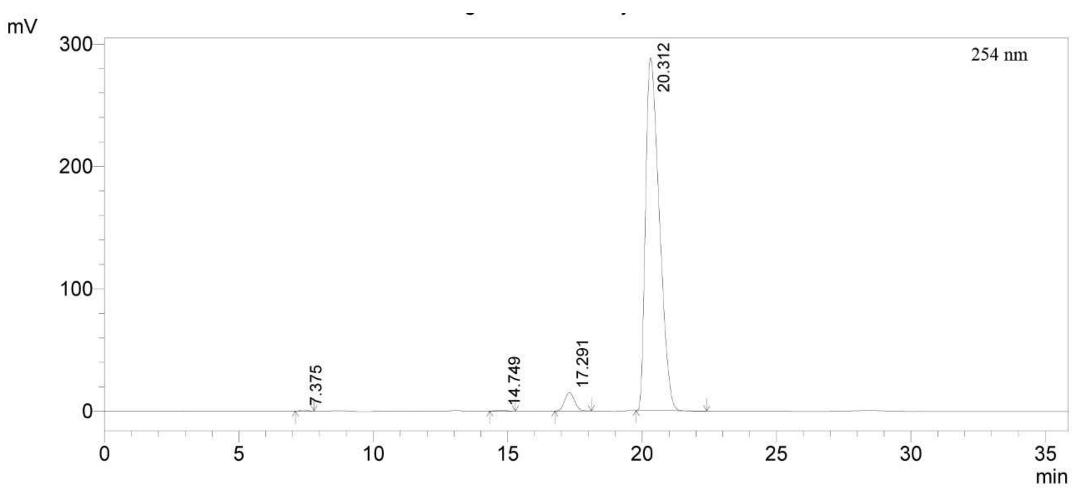

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1	7. 375	14795	0. 139
2	14. 749	11557	0. 108
3	17. 291	386300	3. 619
4	20. 312	10262837	96. 135
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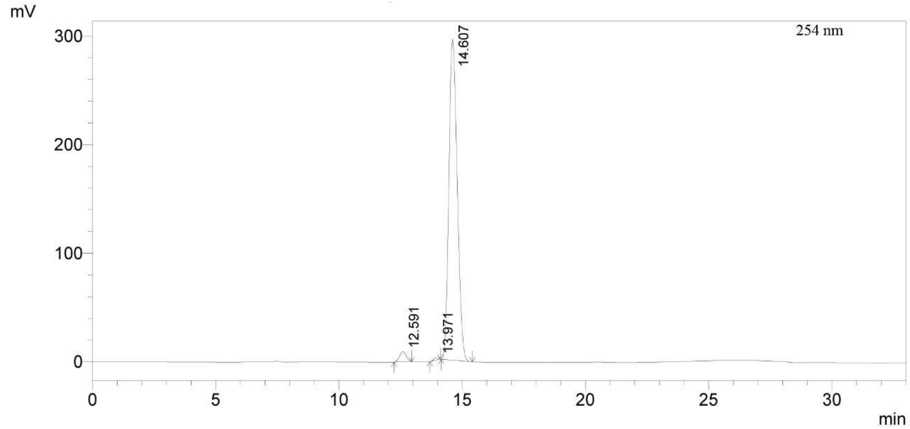

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2	13.971	30754	0.429
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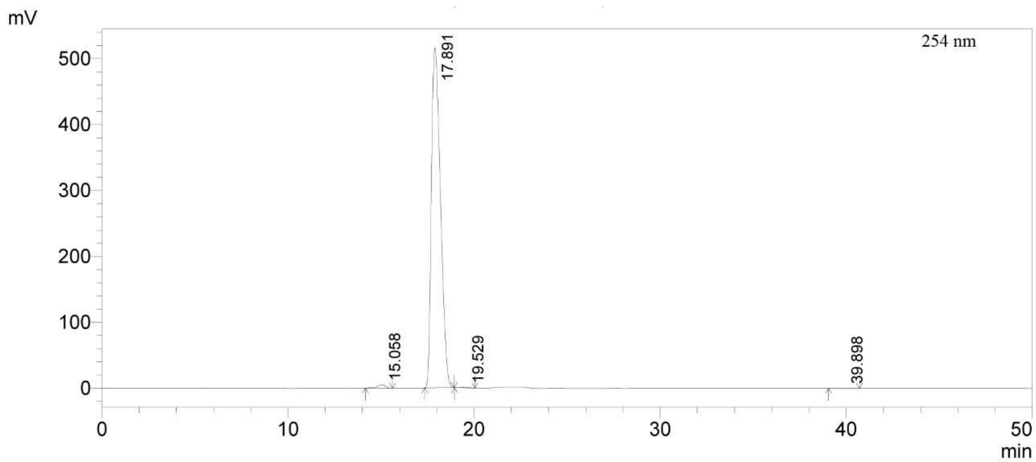

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1	15.058	166413	0.943
2	17.891	17442648	98.855
3	19.529	19136	0.108
4	39.898	16456	0.093
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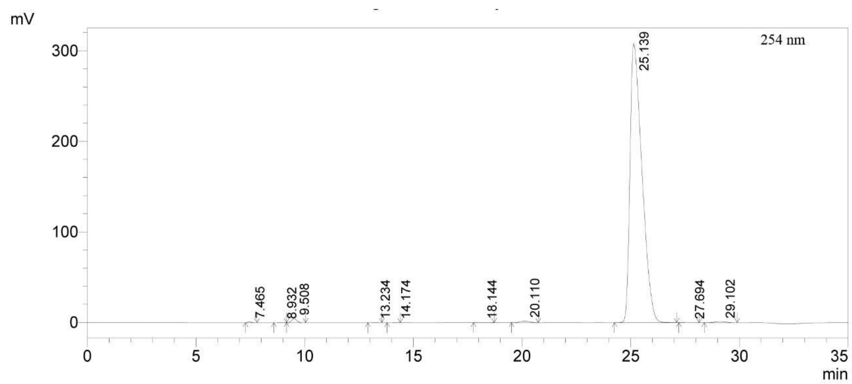

peak#	retention time	area	area %
1	7.465	8305	0.070
2	8.932	2272	0.019
3	9.508	68808	0.576
4	13.234	2799	0.023
5	14.174	1753	0.015
6	18.144	5694	0.048
7	20.110	48154	0.403
8	25.139	11758557	98.501
9	27.694	4674	0.039
10	29.102	36516	0.306
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2.2. FT-IR spectrum

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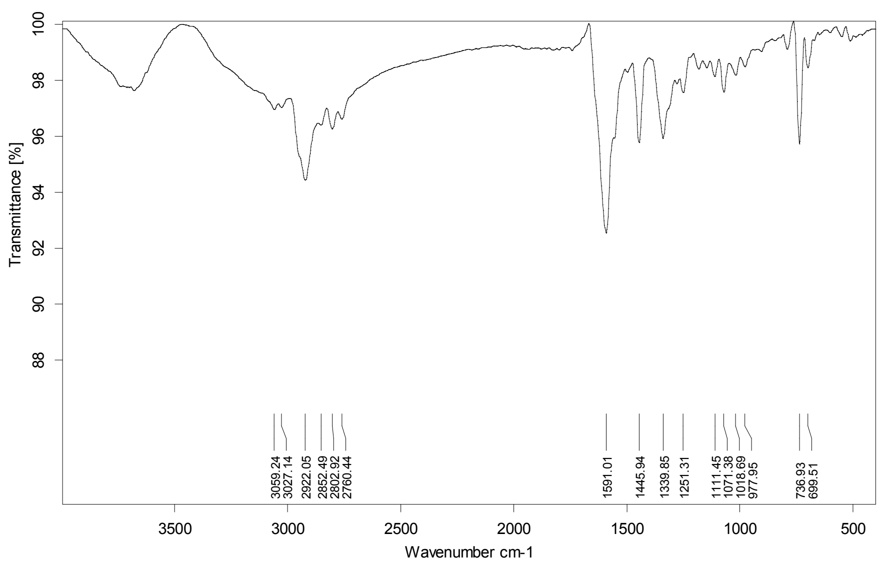

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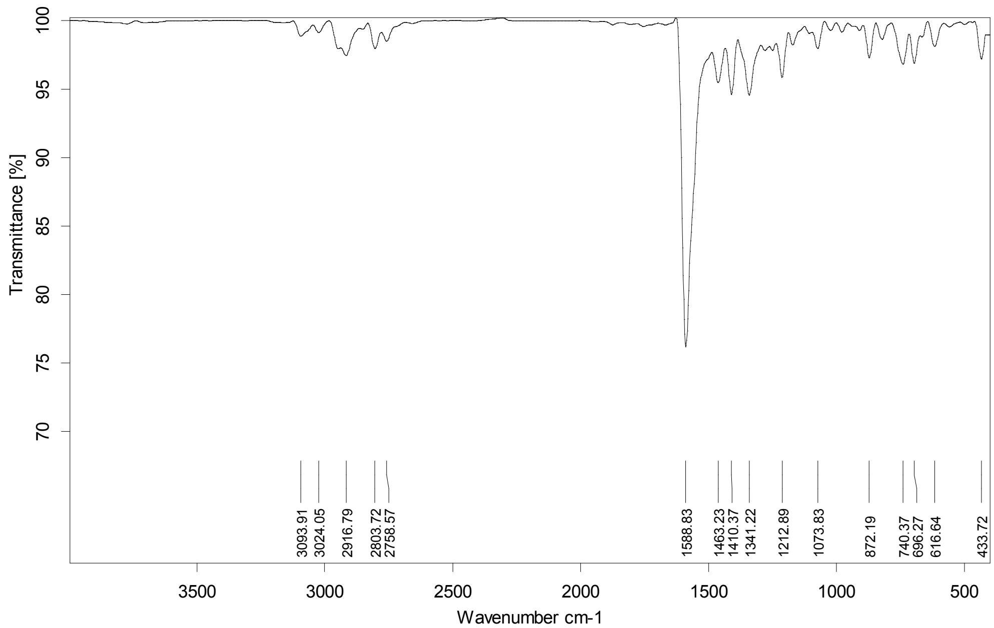

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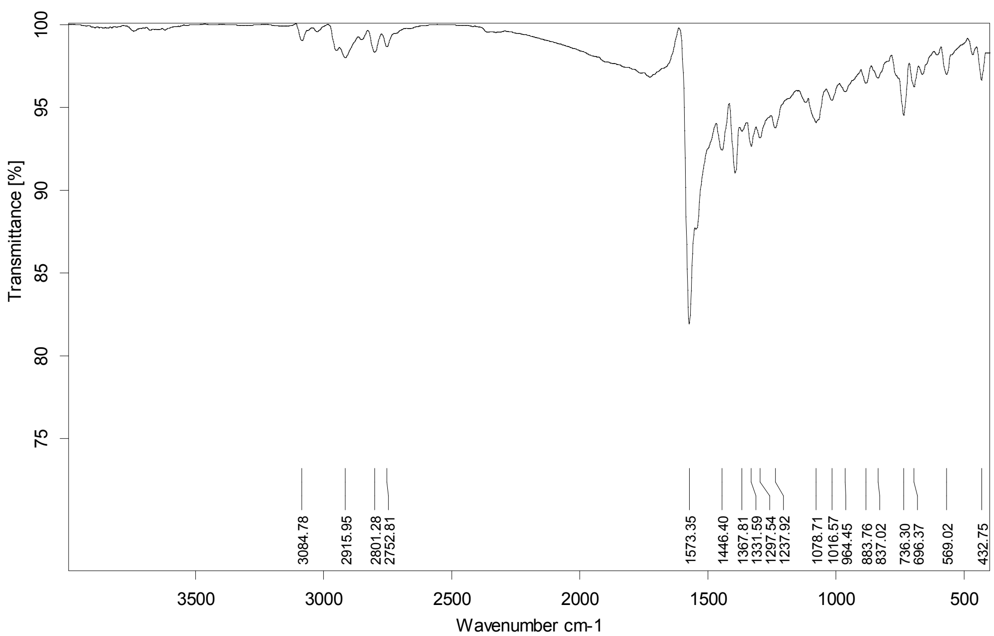

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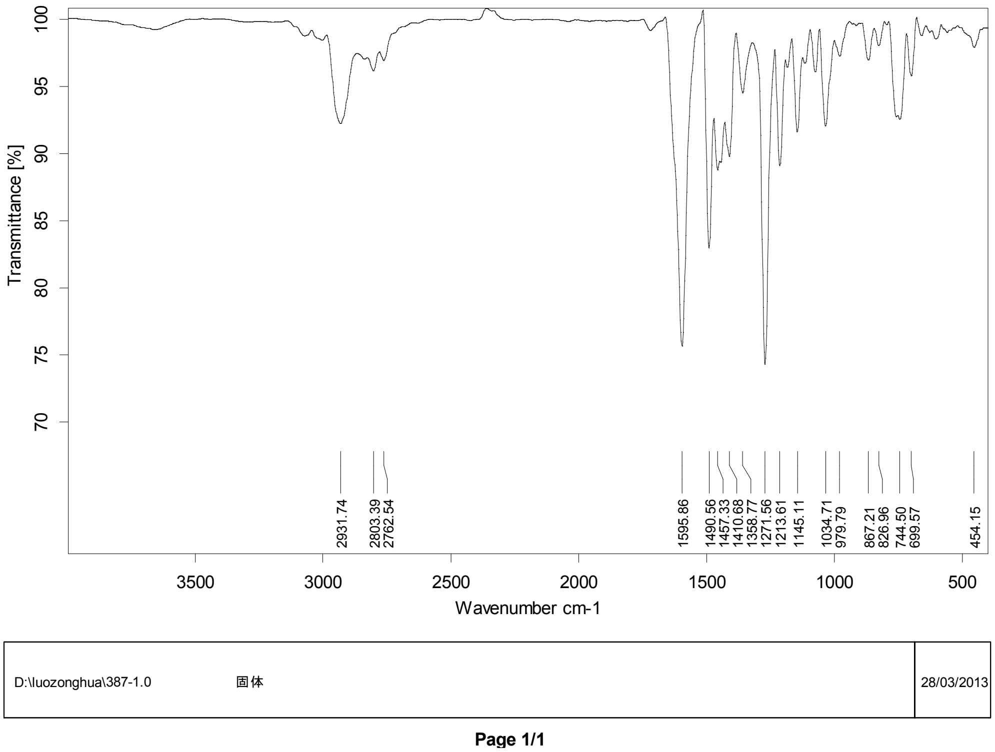
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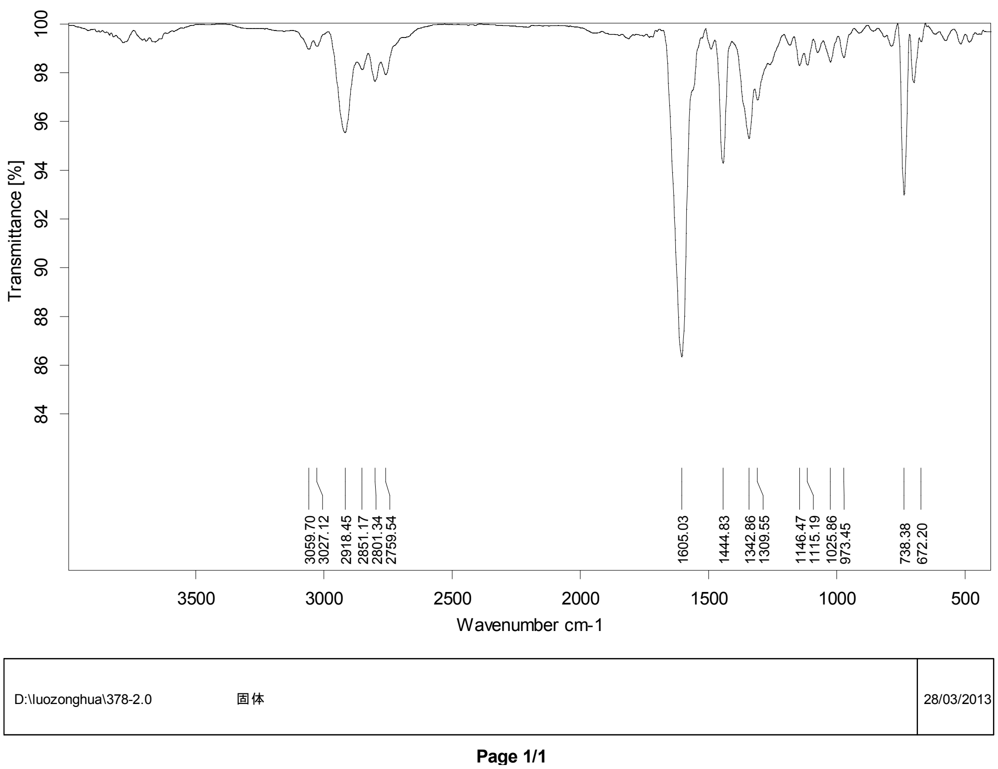
6a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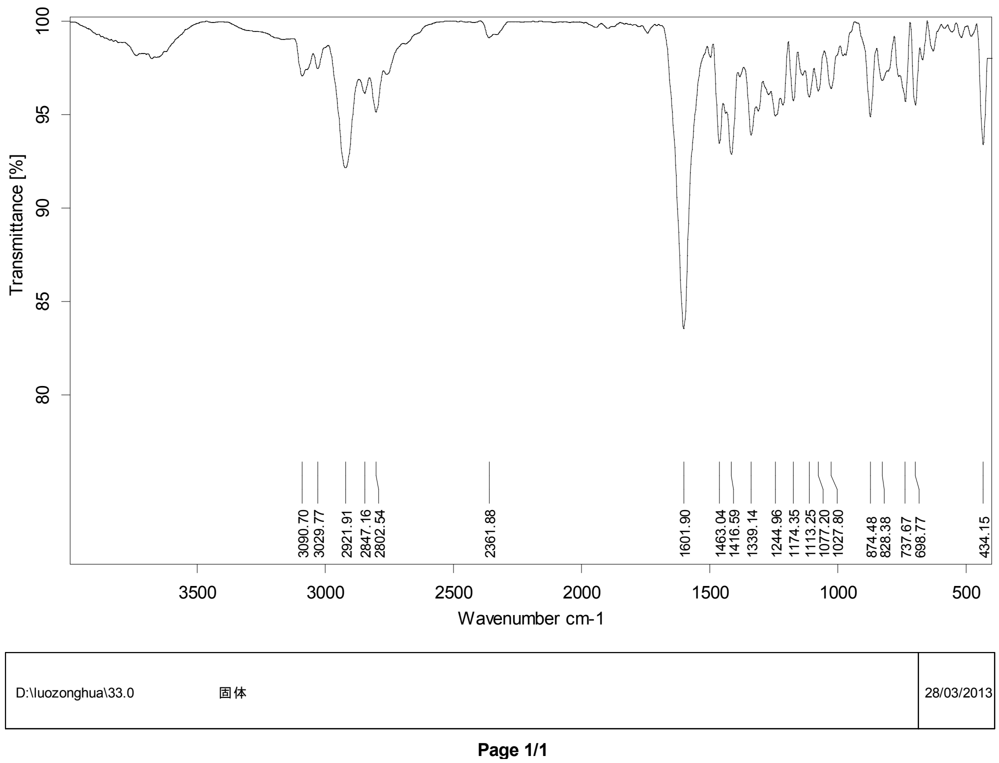
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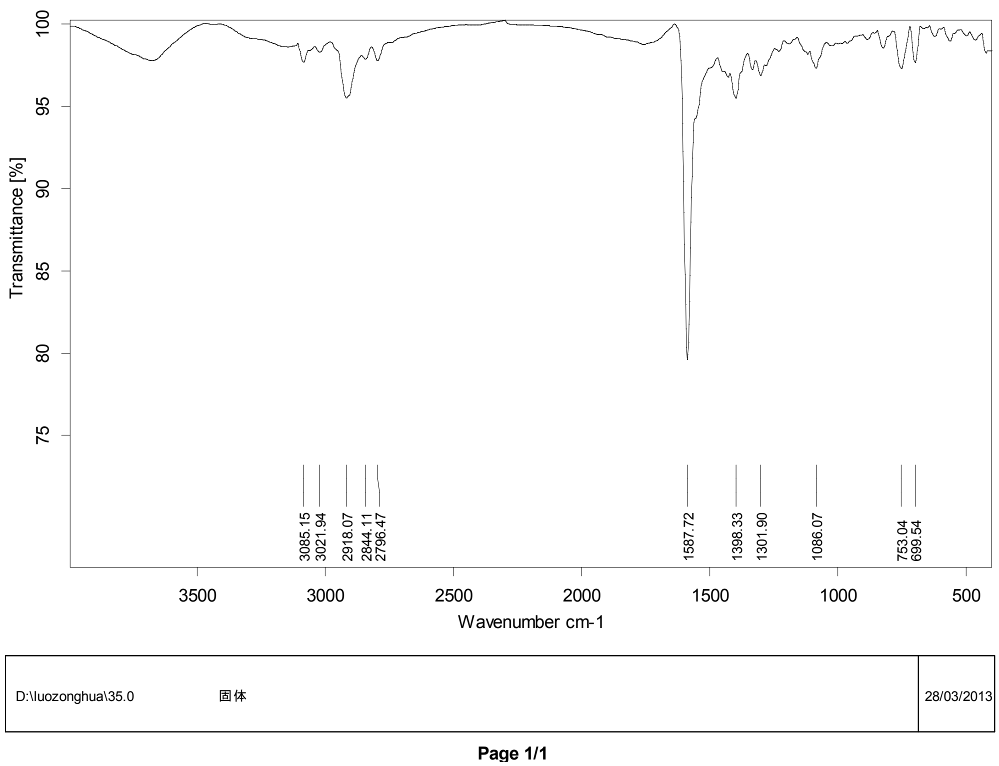
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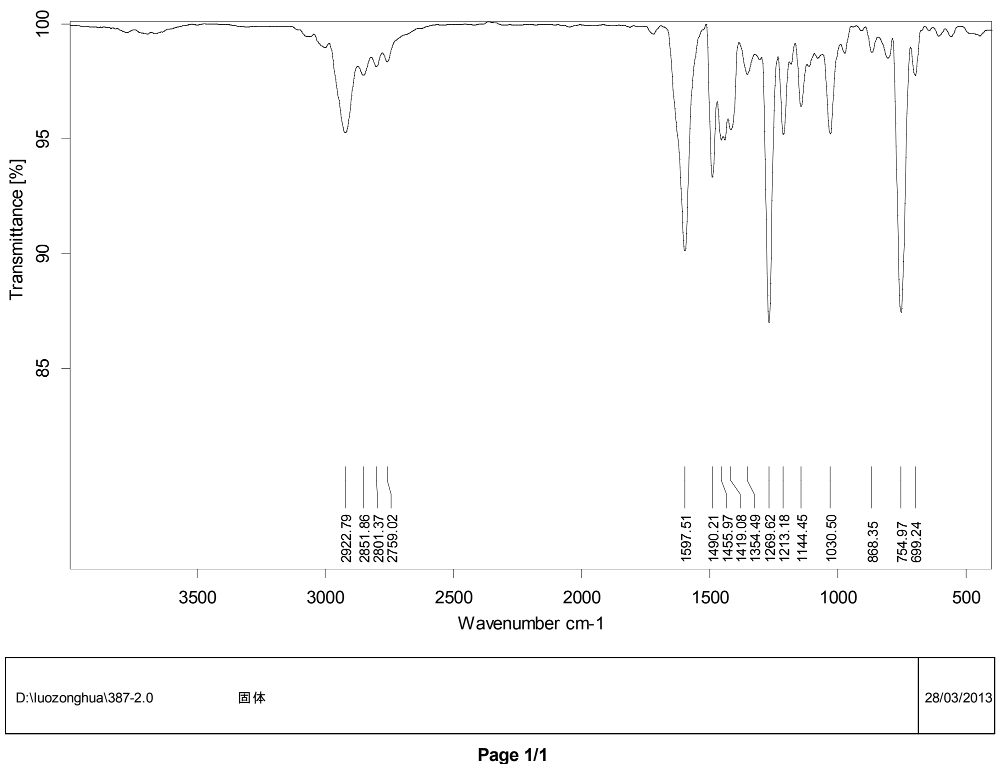
6d



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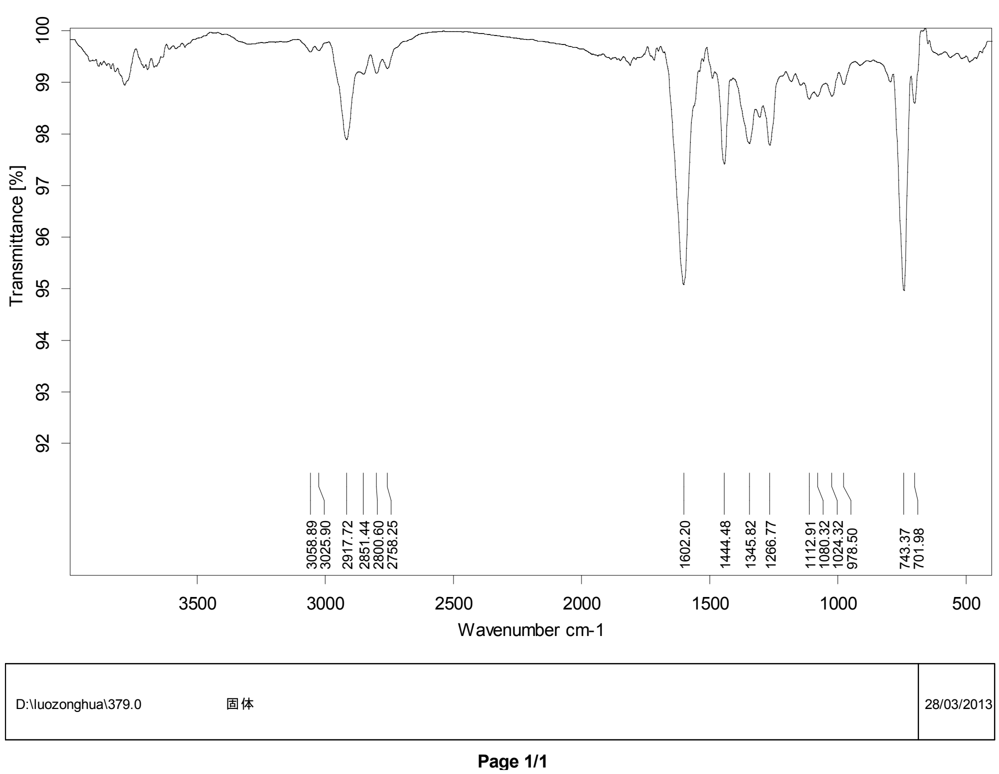
7a



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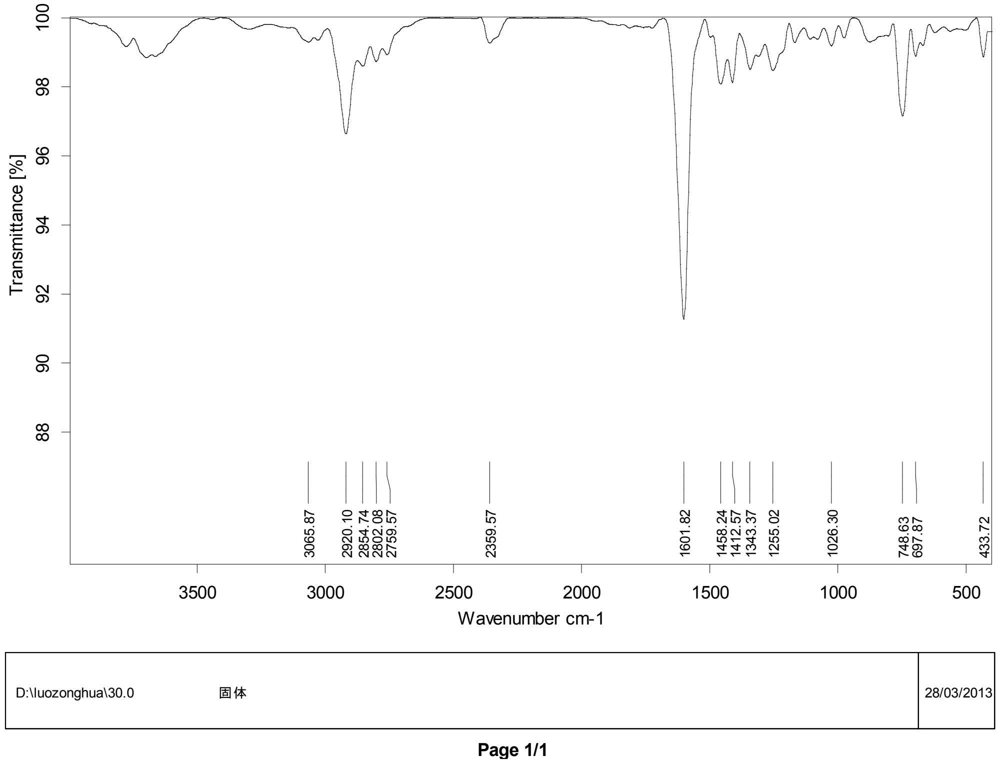
7b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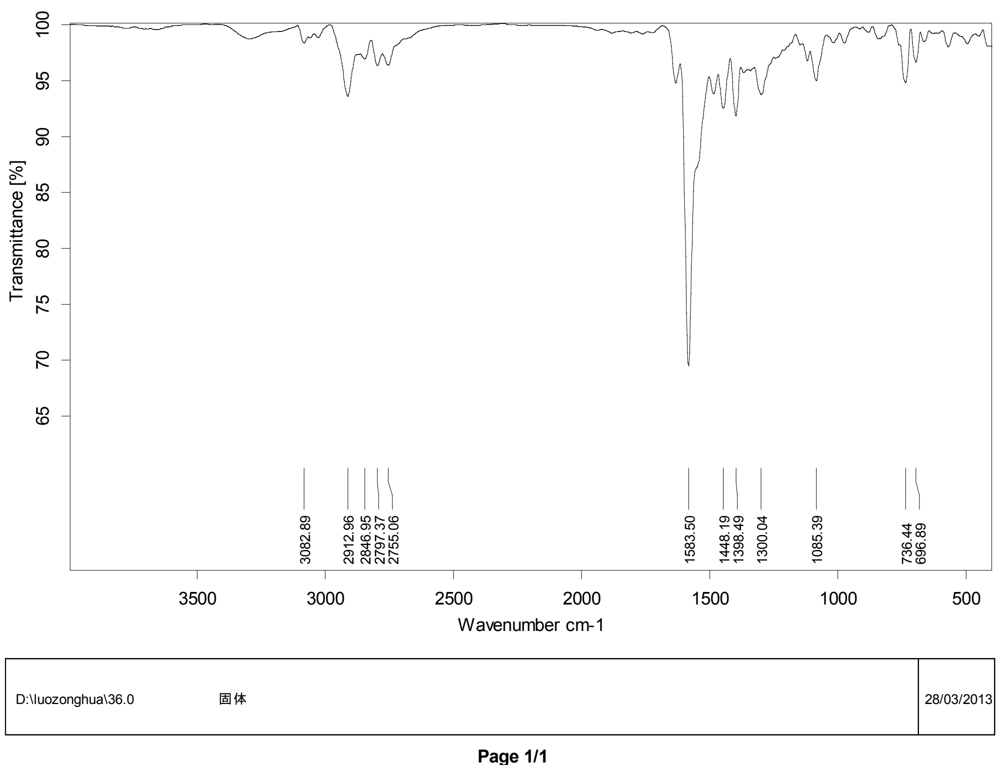
7c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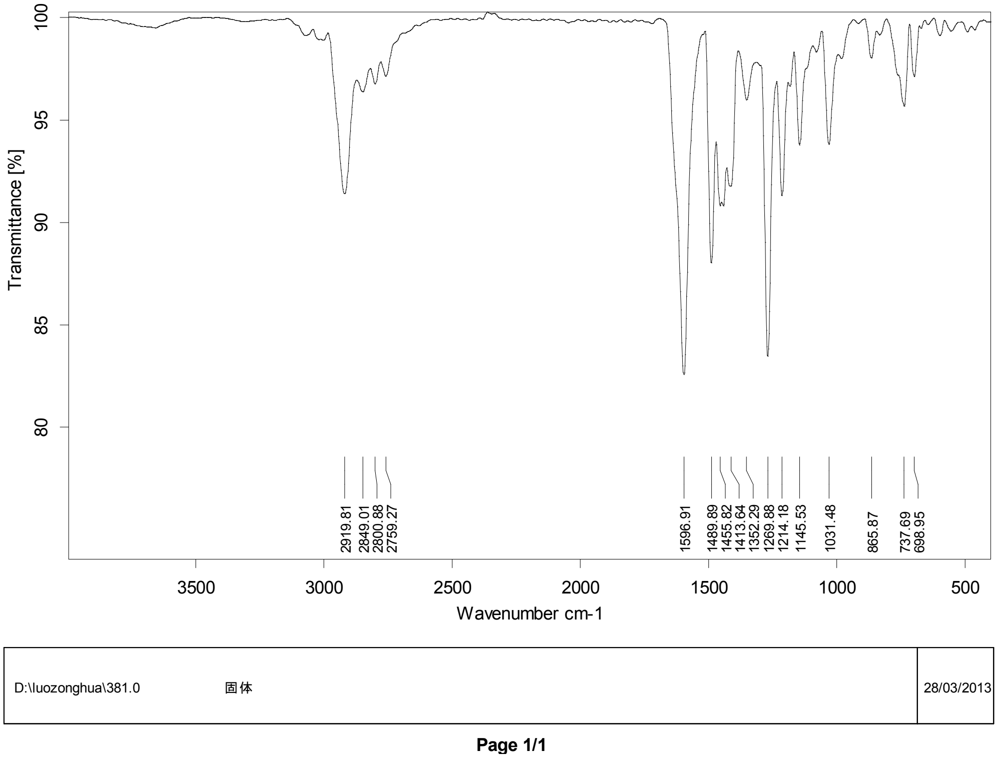
7d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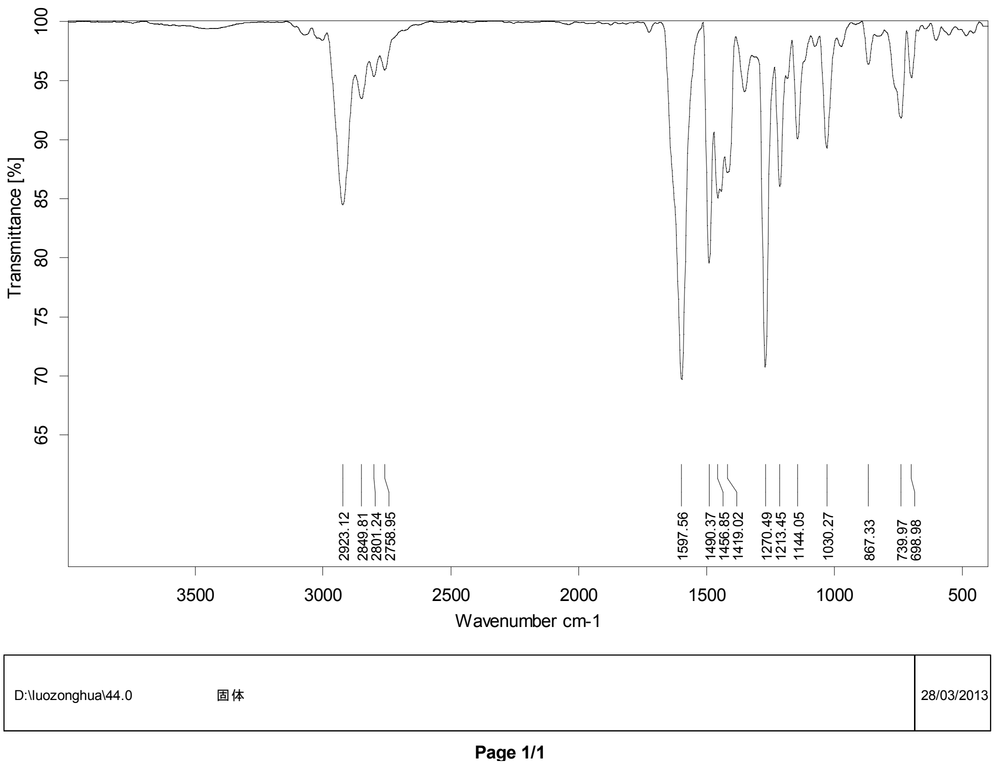
8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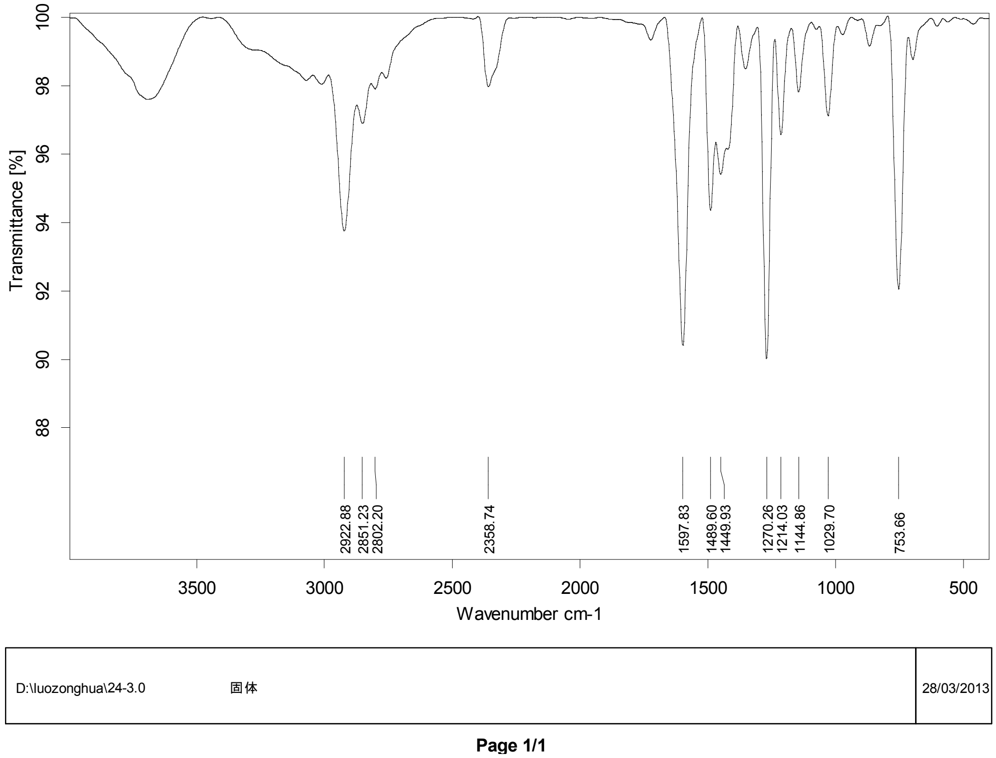
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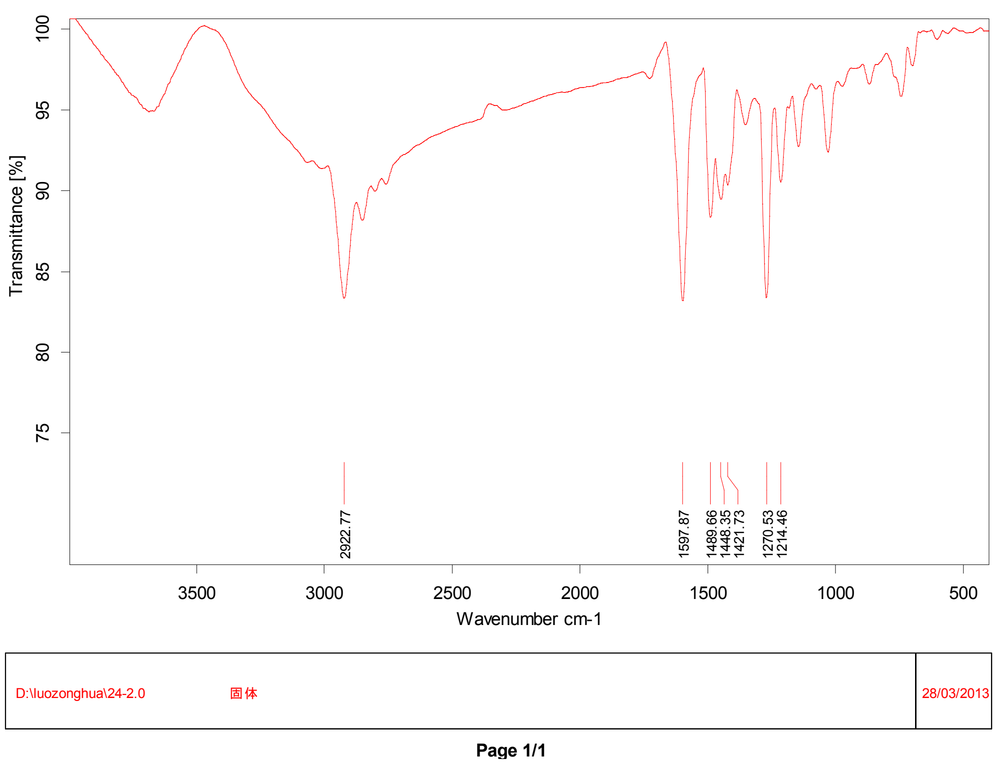
Transmittance [%]
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90
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80
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1214.46
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1
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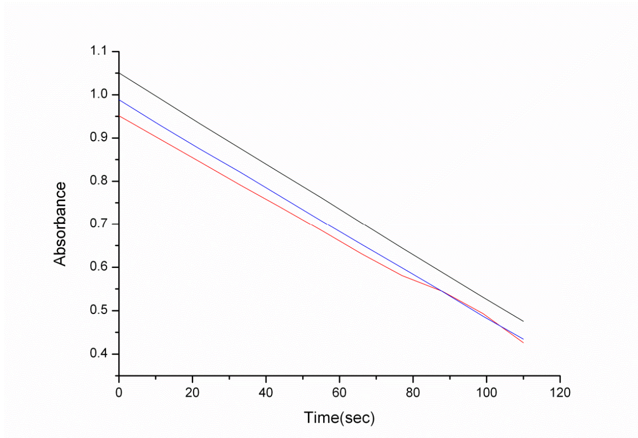
3. Coupled Reductase Assay

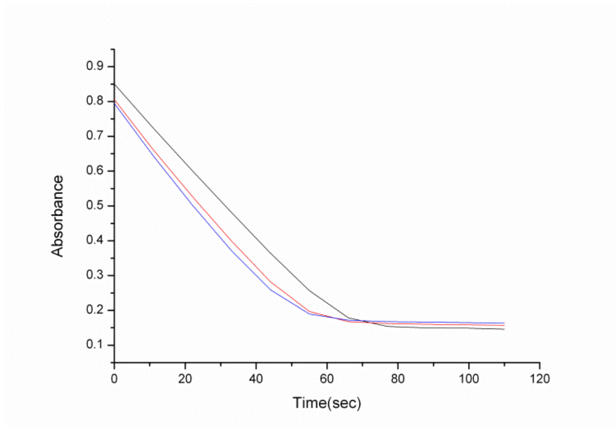
Procedure: Phosphate buffer solution of pH 7.5 was taken in 1 mL cuvette. GSH(2 mM), NADPH(0.4 mM), GR(1.3 U/mL) were added into cuvette contained buffer solution. Finally, H_2O_2(1.6 mM) was added to initiate the reaction in a cuvette having mixture of all and immediately start the experiment for the control values in absence of any catalyst. For the test samples(80 μM), solution was made in MeOH and added into cuvette containing the mixture of buffer solution, GSH, NADPH and GR. Now, H_2O_2 was added to initiate the reaction.

Table S1. Control values (in the absence of the catalyst) in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), and H_2O_2 (1.6 mM), at pH 7.5 in MeOH.



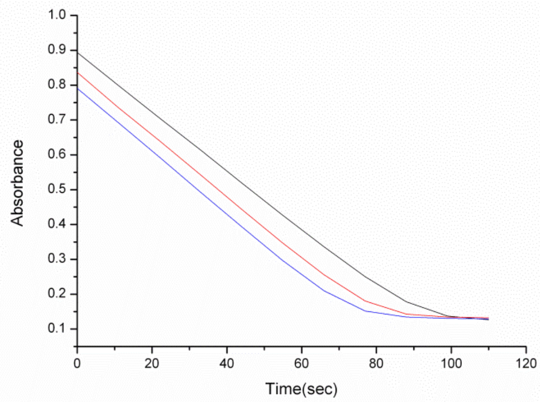
Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.0583	0.318	51.1254	
2	0.053	0.289091	46.47764	49.5±2.6
3	0.0579	0.315818	50.77463	

Table S2. Reduction rate (v_0) of **7d** in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and **7d** (80 μM) , at pH 7.5 in MeOH.



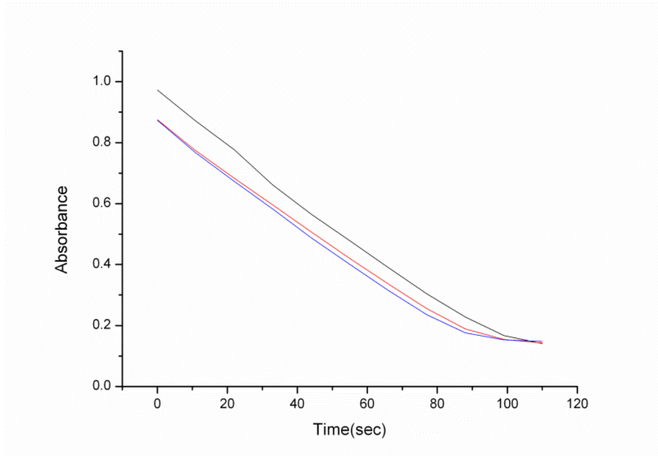
Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.1270	0.6930	111.37	
2	0.1445	0.7882	126.7173	123.5±10.9
3	0.1510	0.8236	132.4174	

Table S3. Reduction rate (v_0) of **8** in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and **8**(80 μM), at pH 7.5 in MeOH.



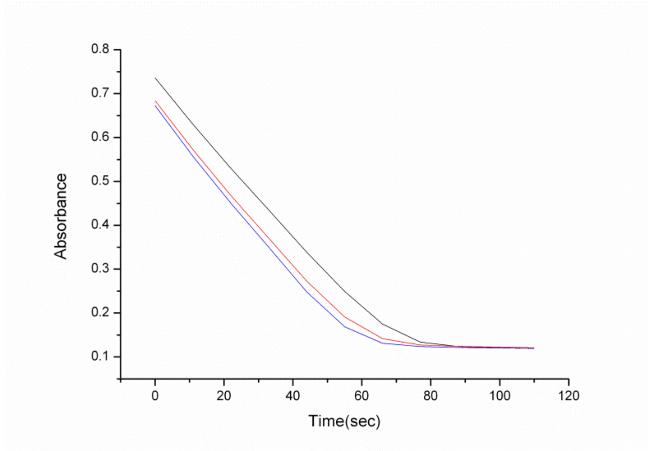
Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.0944	0.514909	82.78281	
2	0.1011	0.551455	88.65829	86.1±3.0
3	0.0989	0.539455	86.72903	

Table S4. Reduction rate (v_0) of **9** in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and **9**(80 μM) , at pH 7.5 in MeOH.



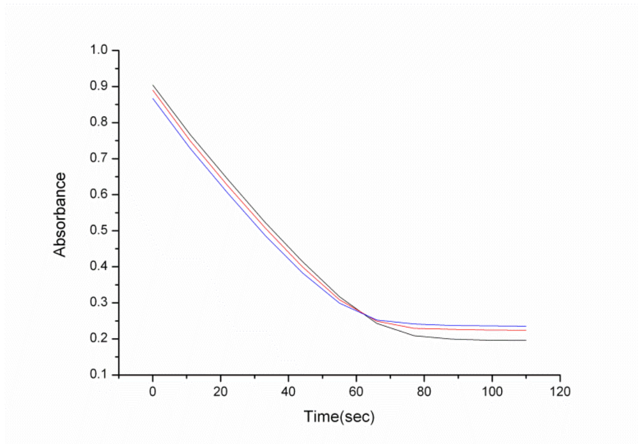
Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.0876	0.477818	76.81964	
2	0.0935	0.51	81.99357	80.1±2.8
3	0.0929	0.506727	81.46741	

Table S5. Reduction rate (v_0) of **10** in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and **10**(80 μM) , at pH 7.5 in MeOH.



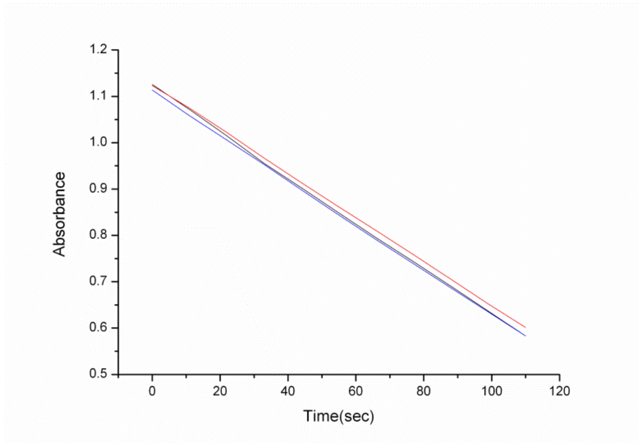
Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.1055	0.575455	92.51681	
2	0.1115	0.608182	97.77843	97.1±4.3
3	0.1152	0.628364	101.0231	

Table S6. Reduction rate (v_0) of ebselen in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and ebselen (80 μM) , at pH 7.5 in MeOH.



Sr. No.	ΔA	ΔA/min	v_0(μM·min^{-1})	v_0(μM·min^{-1})
1	0.1372	0.748364	120.3157	
2	0.1397	0.762	122.508	121.3±1.1
3	0.1382	0.753818	121.1926	

Table S7. Reduction rate (v_0) of donepezil in the coupled reductase assay in the coupled reductase assay. Catalyzed reduction of H_2O_2 by GSH: GSH (2 mM), NADPH (0.4 mM), GR (1.3 unit/mL), H_2O_2 (1.6 mM) and donepezil (80 µM) , at pH 7.5 in MeOH.



Sr. No.	ΔA	ΔA/min	v_0(µM·min^{-1})	v_0(µM·min^{-1})
1	0.054	0.294545	47.35457	
2	0.0484	0.264	42.44373	46.1±3.2
3	0.0553	0.301636	48.49459	

4. Tables of results for the PAMPA

Table S8 Permeability ($P_e \times 10^{-6}$ cm s^{-1}) in the PAMPA-BBB assay for 13 commercial drugs, used in the Experiment Validation.

Commercial drugs	Bibl [a]	PBS : EtOH (70 : 30) [b]
testosterone	17	22.3 ± 1.4
verapamil	16	21.2 ± 1.9
desipramine	12	16.4 ± 1.2
progesterone	9.3	17.7 ± 1.2
promazine	8.8	14.3 ± 0.5
chlorpromazine	6.5	6.0 ± 0.3
clonidine	5.3	5.1 ± 0.3
piroxicam	2.5	0.24 ± 0.01
hydrocortisone	1.9	0.65 ± 0.01
lomefloxacin	1.1	0.37 ± 0.02
atnolol	0.8	0.78 ± 0.02
ofloxacin	0.8	0.37 ± 0.02
theophylline	0.1	0.26 ± 0.01

[a] Taken from reference 4. [b] Data are the mean ± SD of three independent experiments

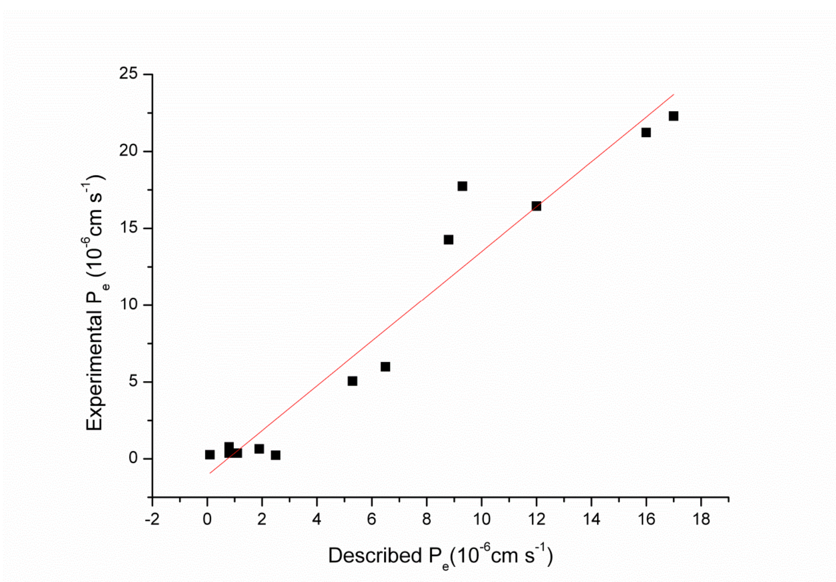


Figure S1. Lineal correlation between experimental and reported permeability of commercial drugs using the PAMPA-BBB assay. P_e (exp.)=1.4574Pe (bibl.) -1.0773 (R^2=0.9427)

Table S9. Ranges of Permeability of PAMPA-BBB Assays (P_e, 10^{-6} cm s^{-1})

Compounds of high BBB permeation (CNS+)	$P_e > 4.7$
Compounds of uncertain BBB permeation (CNS+/-)	$4.7 > P_e > 1.8$
Compounds of low BBB permeation (CNS-)	$P_e < 1.8$

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