## Naturally Occurring Homoisoflavonoids Function as Potent Protein Tyrosine Kinase Inhibitors by c-Src-based High-Throughput Screening

Li-Gen Lin,<sup>†,||</sup> Hua Xie,<sup>‡,||</sup> Hong-Lin Li,<sup>§</sup> Lin-Jiang Tong,<sup>‡</sup> Chun-Ping Tang,<sup>†</sup> Chang-Qiang Ke,<sup>†</sup> Qun-Fang Liu,<sup>†</sup> Li-Ping Lin,<sup>‡</sup> Mei-Yu Geng,<sup>‡</sup> Hua-Liang Jiang,<sup>§</sup> Wei-Min Zhao,<sup>\*,†</sup> Jian Ding,<sup>\*,‡</sup> and Yang Ye<sup>\*,†</sup>

<sup>1</sup>Department of Natural Products Chemistry, Division of Anti-tumor Pharmacology and Center for Drug Discovery and Design, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

## **Supporting Information**

Page 3–21, spectroscopic data of haematoxylol (1), epihaematoxylol (2), 4-*O*-methylhaematoxylol (3), 4-*O*-methylepihaematoxylol (4), haematoxylene (6), haematoxylone (7), isohematoxylin (10), sappanone B (11), sappanchalcone (20), hematoxylol (25) and hematoxylin (26).

Figure S1. <sup>1</sup>H NMR spectrum of haematoxylol (1) in CD<sub>3</sub>OD.

Figure S2. <sup>13</sup>C NMR and DEPT spectra of haematoxylol (1) in CD<sub>3</sub>OD.

Figure S3. LC-MS spectrum of haematoxylol (1).

Figure S4. <sup>1</sup>H NMR spectrum of epihaematoxylol (2) in CD<sub>3</sub>OD.

Figure S5. LC-MS spectrum of epihaematoxylol (2).

Figure S6. <sup>1</sup>H NMR spectrum of 4-O-methylhaematoxylol (**3**) in CD<sub>3</sub>OD.

Figure S7. <sup>13</sup>C NMR and DEPT spectra of 4-*O*-methylhaematoxylol (**3**) in CD<sub>3</sub>OD.

Figure S8. LC-MS spectrum of 4-O-methylhaematoxylol (3).

Figure S9. <sup>1</sup>H NMR spectrum of 4-*O*-methylepihaematoxylol (4) in CD<sub>3</sub>OD.

Figure S10. <sup>13</sup>C NMR and DEPT spectra of 4-*O*-methylepihaematoxylol (4) in CD<sub>3</sub>OD.

<sup>&</sup>lt;sup>1</sup> \* To whom all correspondence should be addressed. Y.Y.: tel, +86 21 50806600x3326; fax, +86 21 50806726; e-mail, yye@mail.shcnc.ac.cn. J.D.: tel, +86 21 5080660x2423; fax, +86 21 50806722; e-mail, jding@mail.shcnc.ac.cn. W.-M.Z.: tel & fax: +86 21 50806052; e-mail, wmzhao@mail.shcnc.ac.cn.

<sup>&</sup>lt;sup>†</sup> Department of Natural Products Chemistry.

<sup>&</sup>lt;sup>‡</sup> Division of Anti-tumor Pharmacology.

<sup>&</sup>lt;sup>§</sup>Center for Drug Discovery and Design.

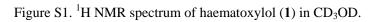
<sup>&</sup>lt;sup>II</sup> Those authors contributed equally.

- Figure S11. LC-MS spectrum of 4-O-methylepihaematoxylol (4).
- Figure S12. <sup>1</sup>H NMR spectrum of haematoxylene (6) in CD<sub>3</sub>OD.
- Figure S13. <sup>13</sup>C NMR and DEPT spectrum of haematoxylene (6) in CD<sub>3</sub>OD.
- Figure S14. LC-MS spectrum of haematoxylene (6).
- Figure S15. <sup>1</sup>H NMR spectrum of haematoxylone (7) in CD<sub>3</sub>OD.
- Figure S16. <sup>13</sup>C NMR and DEPT spectrum of haematoxylone (7) in CD<sub>3</sub>OD.
- Figure S17. LC-MS spectrum of haematoxylone (7).
- Figure S18. <sup>1</sup>H NMR spectrum of isohematoxylin (10) in CD<sub>3</sub>COCD<sub>3</sub>.
- Figure S19. <sup>13</sup>C NMR and DEPT spectrum of isohematoxylin (10) in CD<sub>3</sub>COCD<sub>3</sub>.
- Figure S20. LC-MS spectrum of isohematoxylin (10).
- Figure S21. <sup>1</sup>H NMR spectrum of sappanone B (11) in CD<sub>3</sub>COCD<sub>3</sub>.
- Figure S22. LC-MS spectrum of sappanone B (11).
- Figure S23. <sup>1</sup>H NMR spectrum of sappanchalcone (**20**) in CD<sub>3</sub>COCD<sub>3</sub>.
- Figure S24. LC-MS spectrum of sappanchalcone (20).
- Figure S25. <sup>1</sup>H NMR spectrum of hematoxylol (**25**) in CD<sub>3</sub>OD.
- Figure S26. LC-MS spectrum of hematoxylol (25).
- Figure S27. <sup>1</sup>H NMR spectrum of hematoxylin (26) in CD<sub>3</sub>COCD<sub>3</sub>.
- Figure S28. LC-MS spectrum of hematoxylin (26).

## Page 22–32: Purity checks for the potent protein tyrosine kinase inhibitors 1–4, 6, 7, 10, 11, 20, 25 and 26 by HPLC with two different mobile phases (CH<sub>3</sub>CN-H<sub>2</sub>O, 2%~25% in 9 min /CH<sub>3</sub>OH-H<sub>2</sub>O, 5%~40% in 15 min).

Figure S29. HPLC purity checks of compound 1.

- Figure S30. HPLC purity checks of compound 2.
- Figure S31. HPLC purity checks of compound **3**.
- Figure S32. HPLC purity checks of compound 4.
- Figure S33. HPLC purity checks of compound 6.
- Figure S34. HPLC purity checks of compound 7.
- Figure S35. HPLC purity checks of compound 10.
- Figure S36. HPLC purity checks of compound 11.
- Figure S37. HPLC purity checks of compound 20.
- Figure S38. HPLC purity checks of compound 25.
- Figure S39. HPLC purity checks of compound 26.



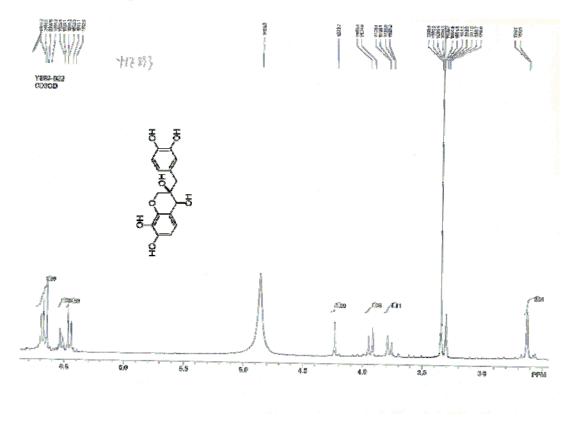
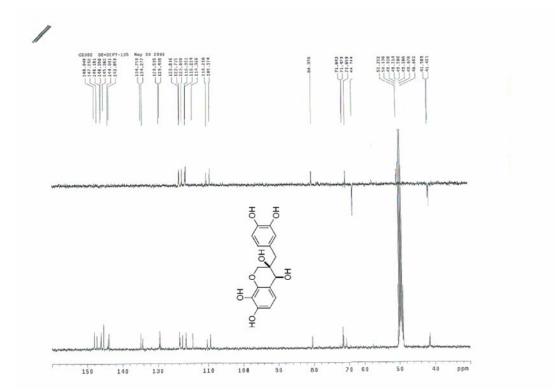


Figure S2. <sup>13</sup>C NMR and DEPT spectra of haematoxylol (1) in CD<sub>3</sub>OD.



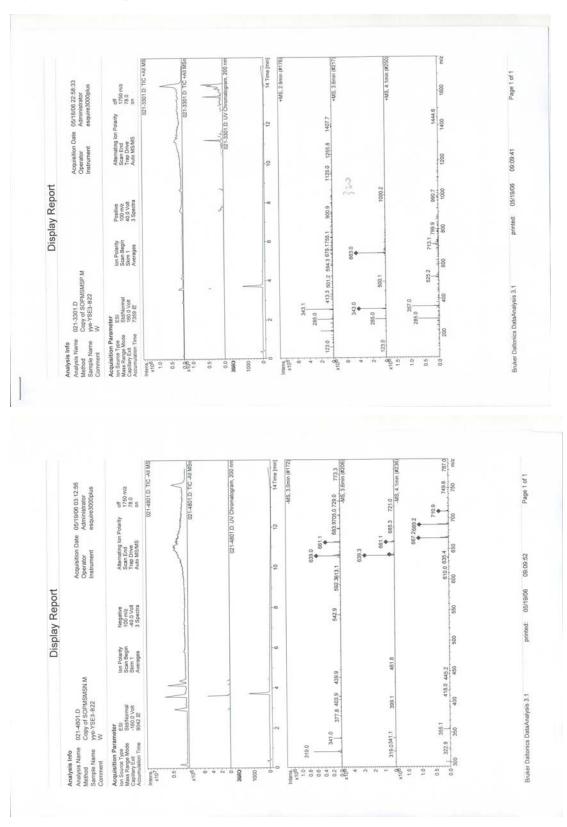


Figure S3. LC-MS spectrum of haematoxylol (1).

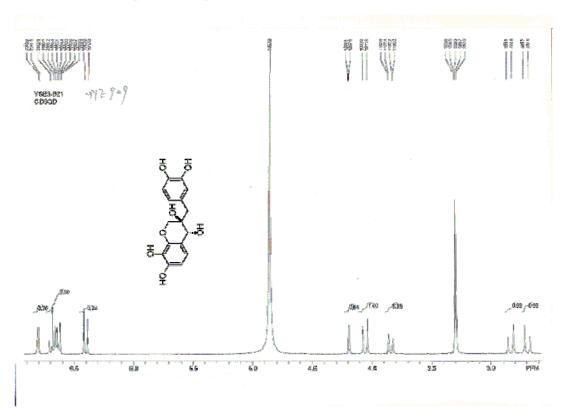
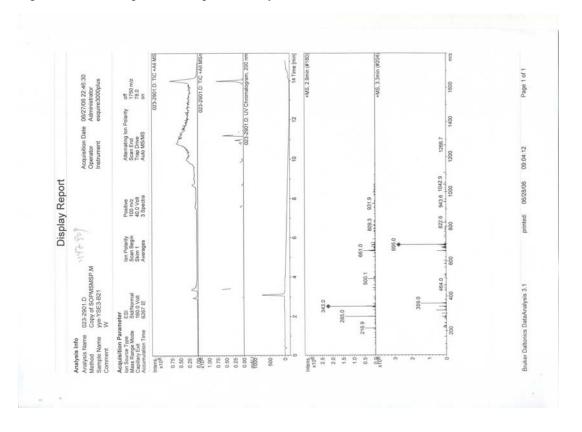


Figure S4. <sup>1</sup>H NMR spectrum of epihaematoxylol (2) in CD<sub>3</sub>OD.

Figure S5. LC-MS spectrum of epihaematoxylol (2).



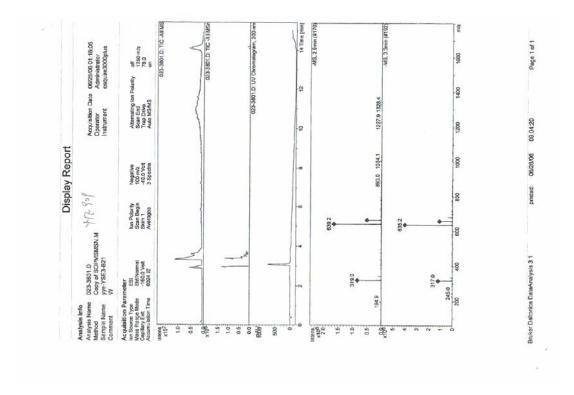
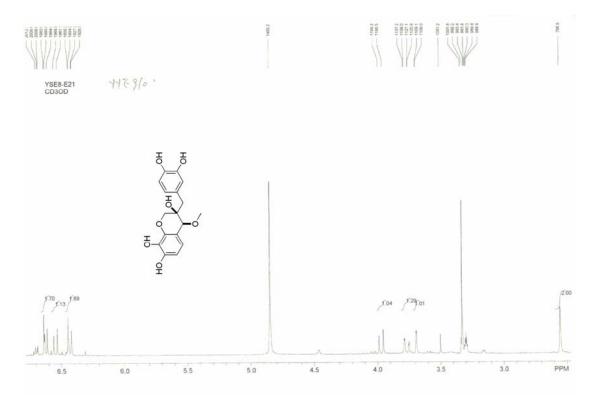


Figure S6. <sup>1</sup>H NMR spectrum of 4-*O*-methylhaematoxylol (**3**) in CD<sub>3</sub>OD.



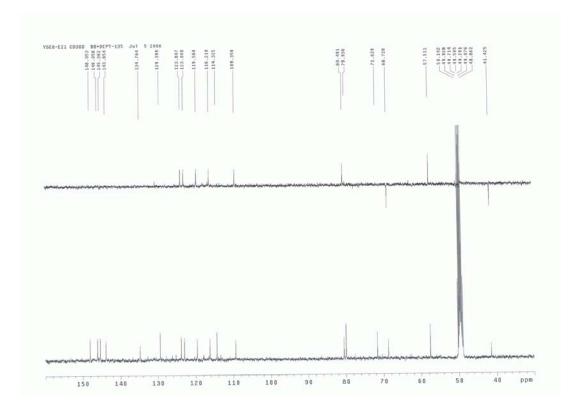


Figure S7. <sup>13</sup>C NMR and DEPT spectra of 4-*O*-methylhaematoxylol (**3**) in CD<sub>3</sub>OD.

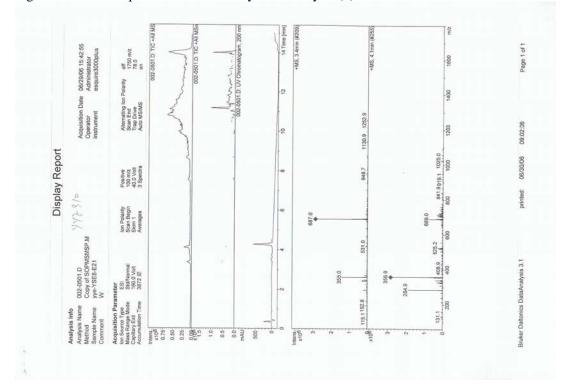


Figure S8. LC-MS spectrum of 4-*O*-methylhaematoxylol (3).

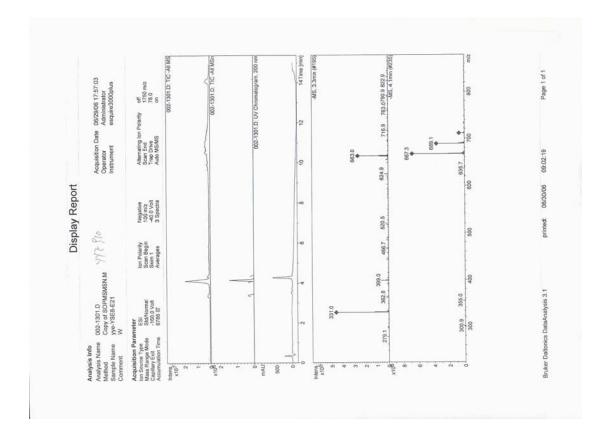
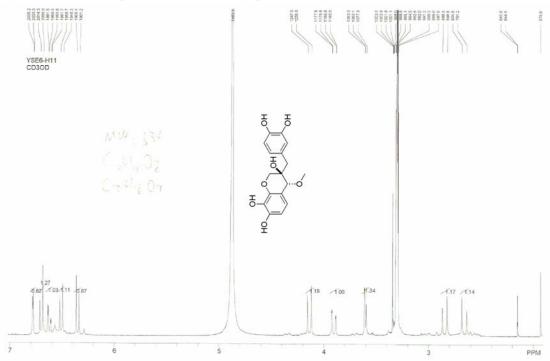


Figure S9. <sup>1</sup>H NMR spectrum of 4-*O*-methylepihaematoxylol (4) in CD<sub>3</sub>OD.



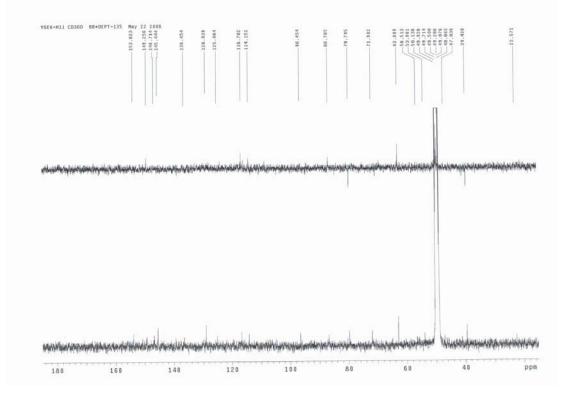
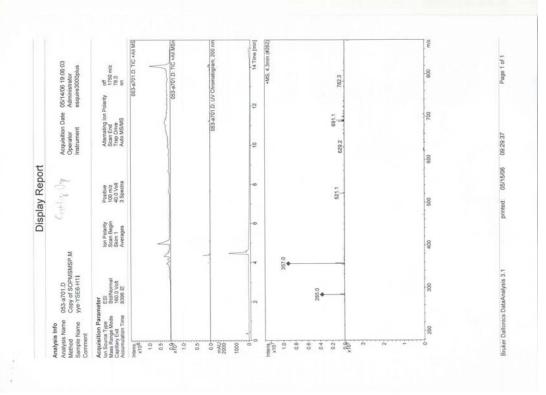
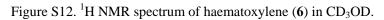
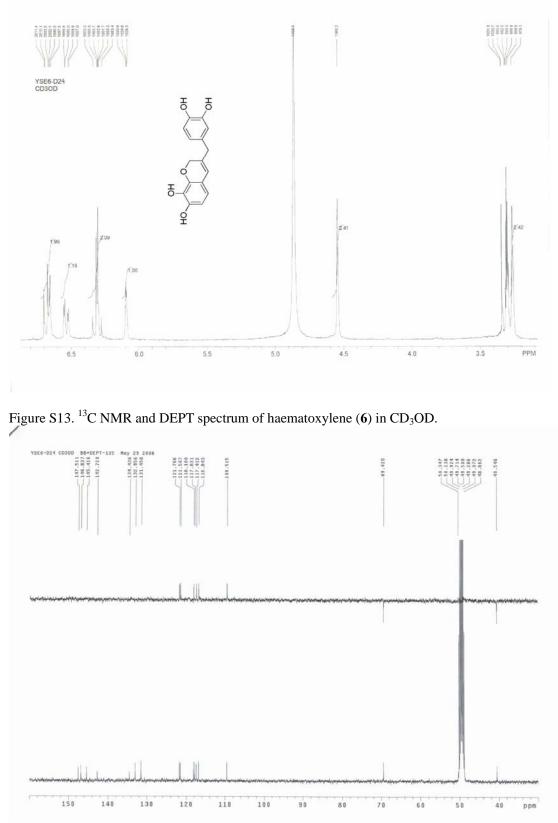


Figure S10. <sup>13</sup>C NMR and DEPT spectra of 4-*O*-methylepihaematoxylol (4) in CD<sub>3</sub>OD.

Figure S11. LC-MS spectrum of 4-O-methylepihaematoxylol (4).







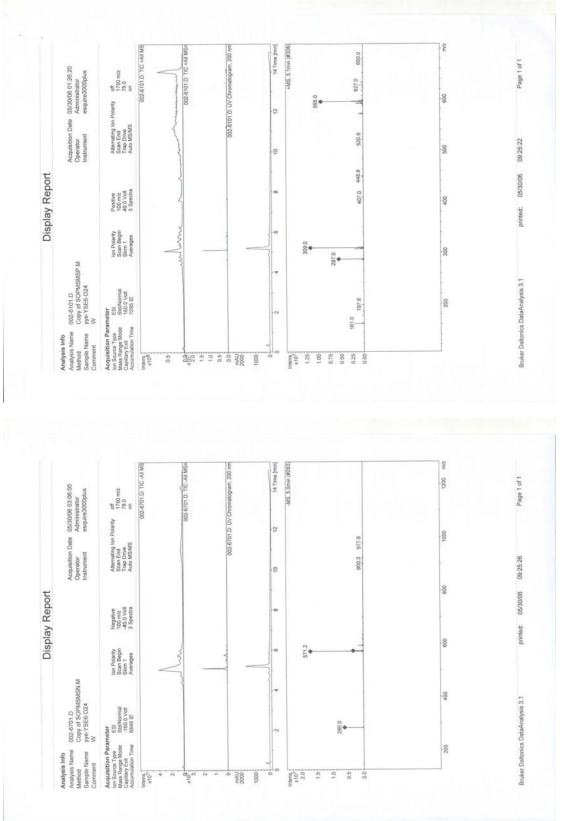


Figure S14. LC-MS spectrum of haematoxylene (6).

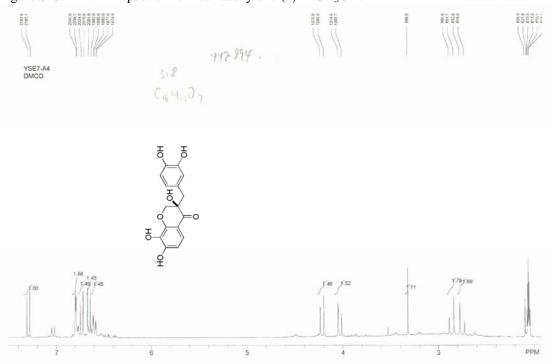
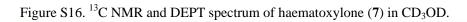
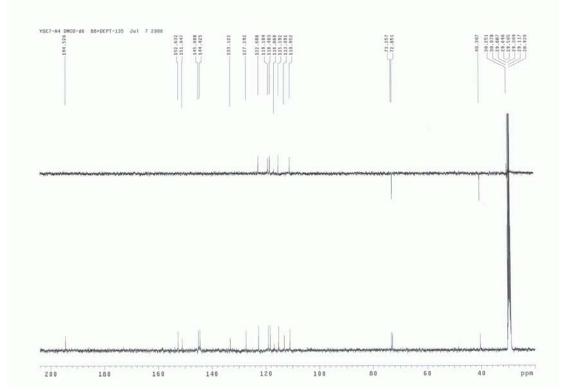


Figure S15. <sup>1</sup>H NMR spectrum of haematoxylone (7) in CD<sub>3</sub>OD.





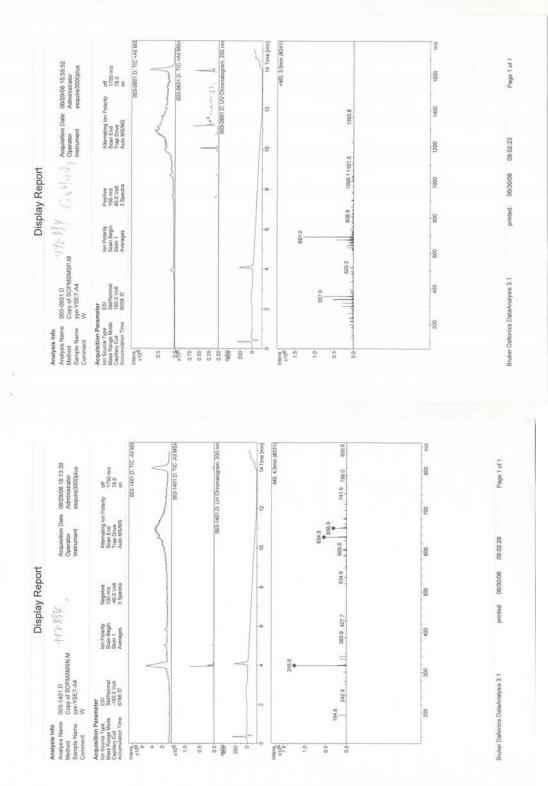


Figure S17. LC-MS spectrum of haematoxylone (7).

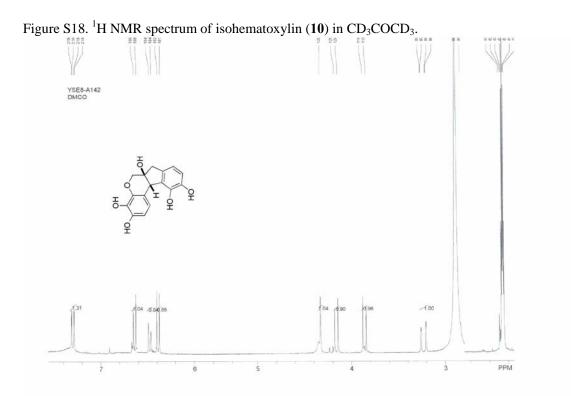
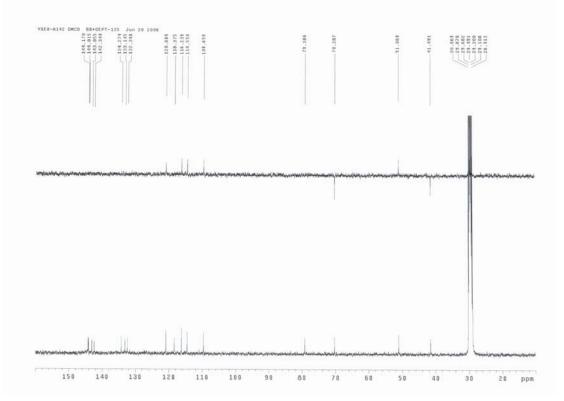
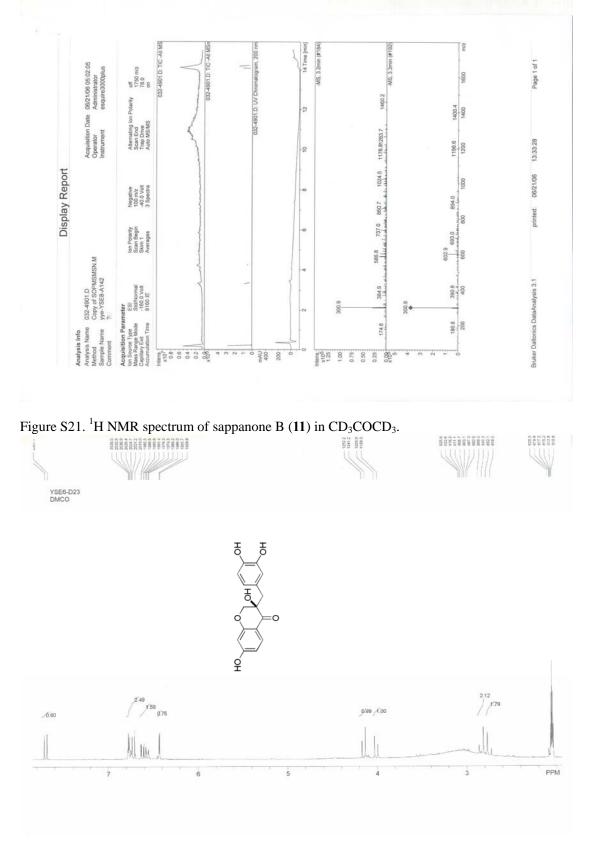


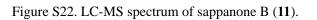
Figure S19. <sup>13</sup>C NMR and DEPT spectrum of isohematoxylin (10) in CD<sub>3</sub>COCD<sub>3</sub>.

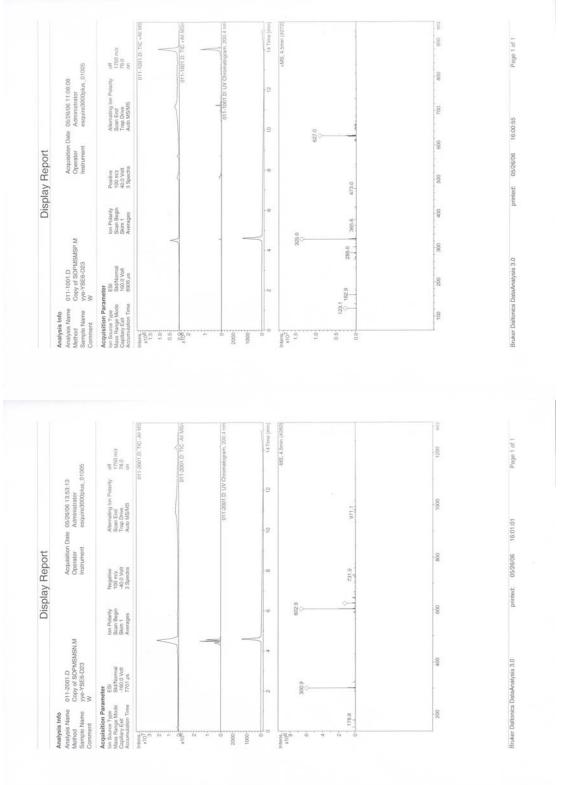


## 14

Figure S20. LC-MS spectrum of isohematoxylin (10).







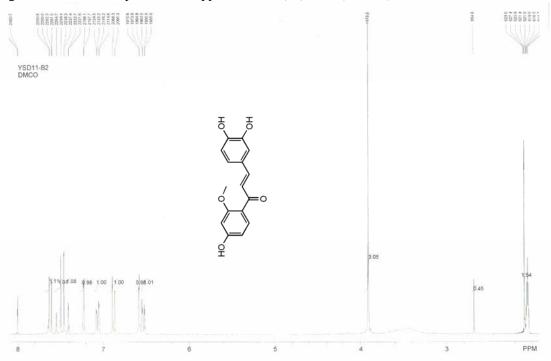
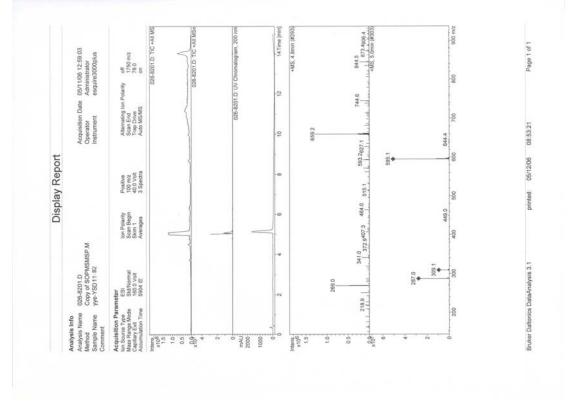


Figure S23. <sup>1</sup>H NMR spectrum of sappanchalcone (**20**) in CD<sub>3</sub>COCD<sub>3</sub>.

Figure S24. LC-MS spectrum of sappanchalcone (20).



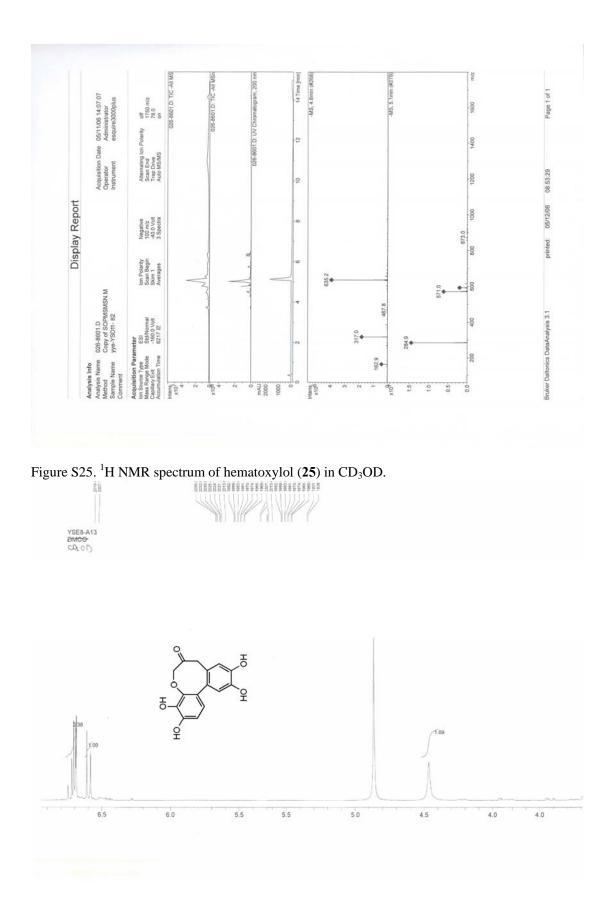
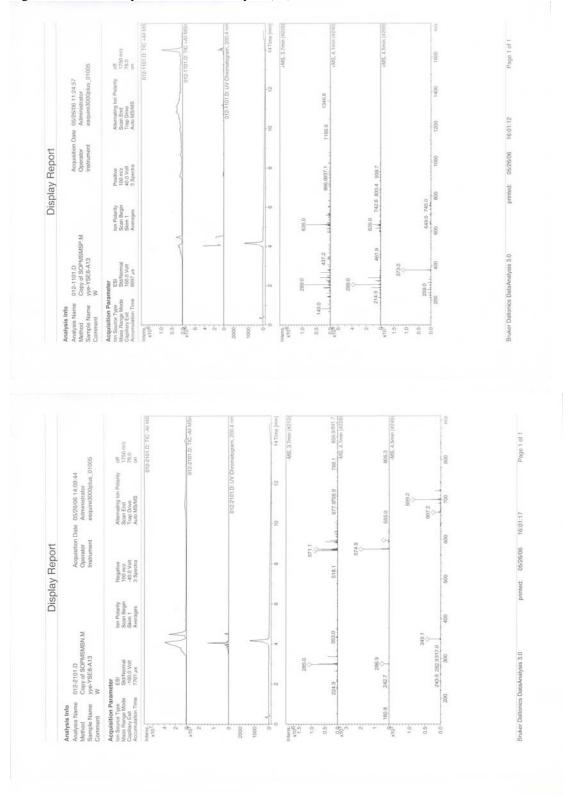


Figure S26. LC-MS spectrum of hematoxylol (25).



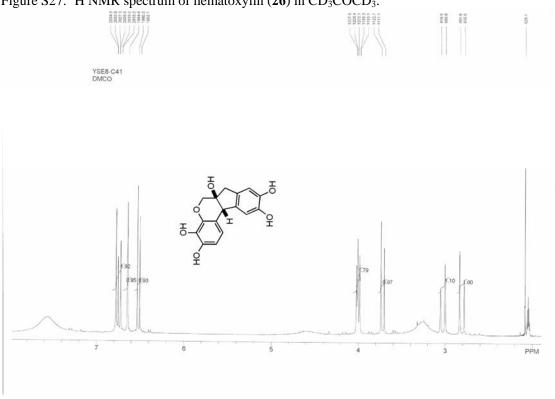
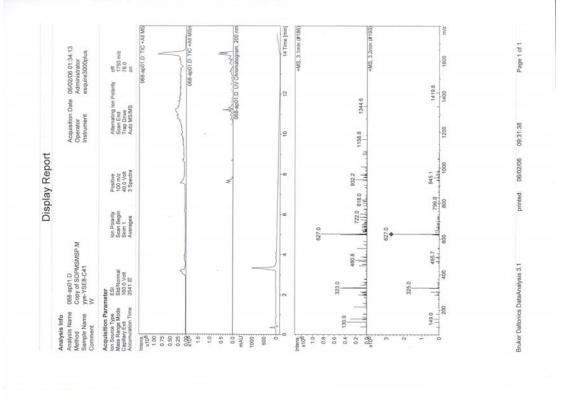
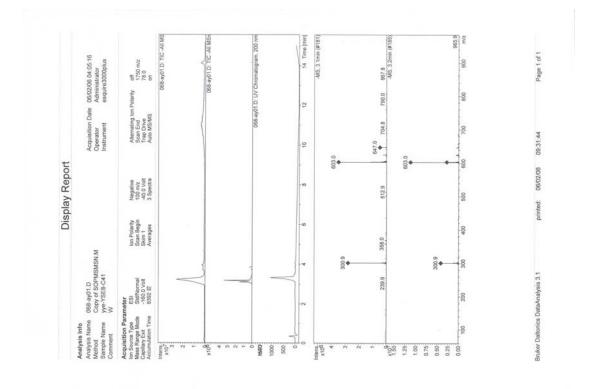


Figure S27. <sup>1</sup>H NMR spectrum of hematoxylin (**26**) in CD<sub>3</sub>COCD<sub>3</sub>.

Figure S28. LC-MS spectrum of hematoxylin (26).





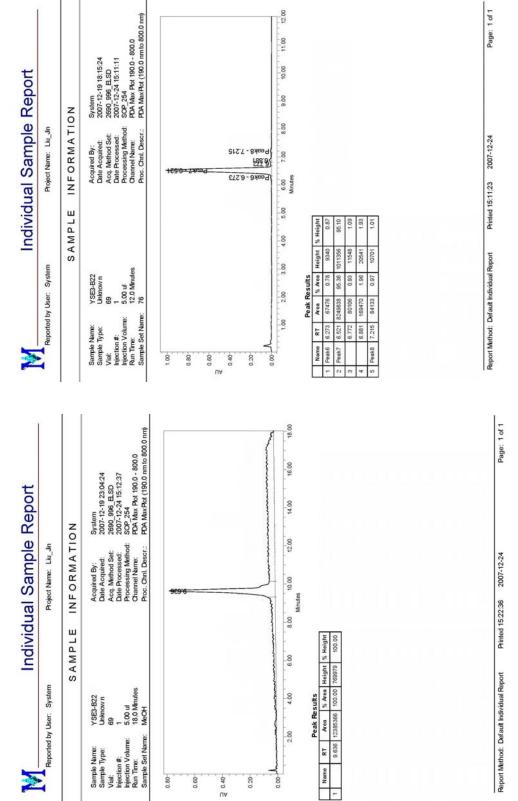
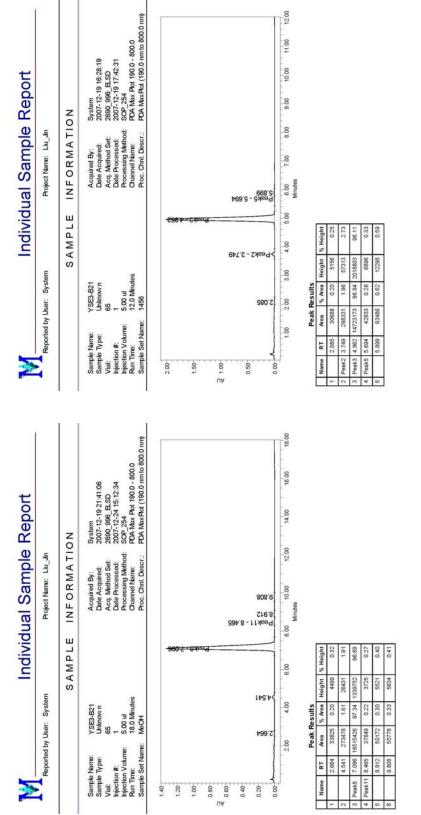


Figure S29. HPLC purity checks of compound 1.



Page: 1 of 1

2007-12-24

Printed 15:06:49

Report Method: Default Individual Report

Page: 1 of 1

2007-12-24

Printed 15:17:02

Report Method: Default Individual Report

Figure S30. HPLC purity checks of compound 2.

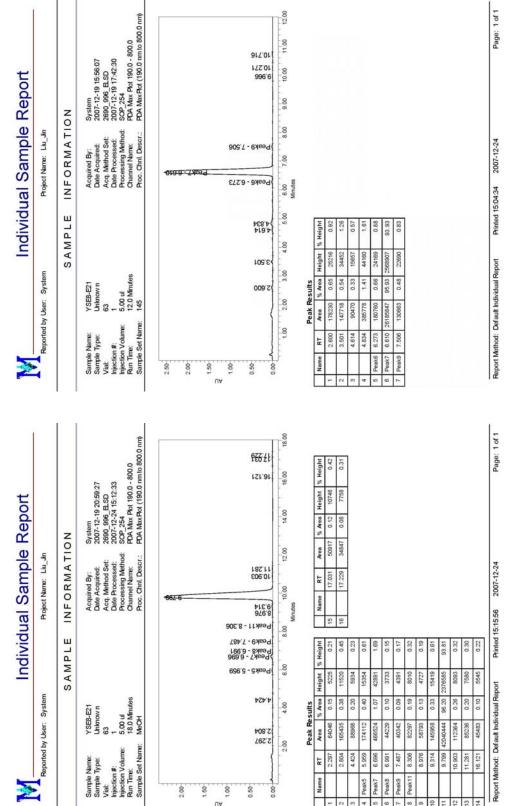


Figure S31. HPLC purity checks of compound **3**.

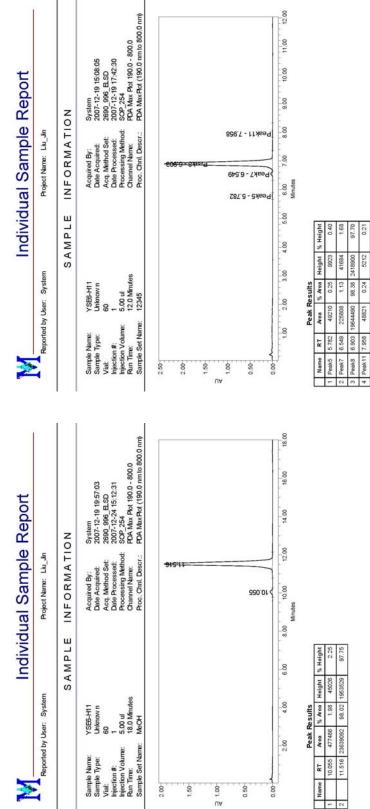


Figure S32. HPLC purity checks of compound 4.

Page: 1 of 1

Report Method: Default Individual Report Printed 14:59:27 2007-12-24

Report Method: Default Individual Report Printed 15:13:59 2007-12-24

Page: 1 of 1

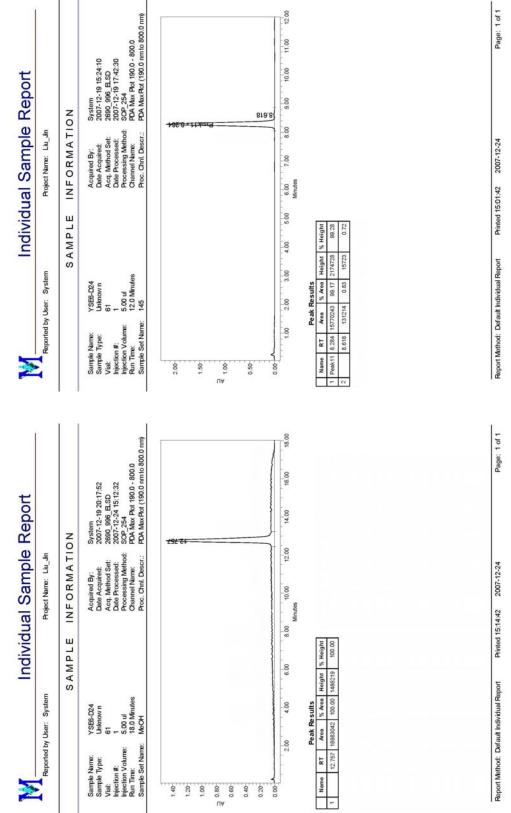


Figure S33. HPLC purity checks of compound 6.

Sample kame: YSE7-A4 Sample kame: YSE7-A4 Viat: Sample kame: YSE7-A4 Viat: Childrown 62 hijection #: 1 Peakt5 - 5842 Peakt5 - 5842 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,885 Peakt 0-7,895 Peakt 0-7,995 Peakt 0-7,9	VFORMATION Acquired By: System Acquired By: System Date Acquired By: 2007-12-19 20:38-40 Acquired By: 2007-12-19 20:38-40 Acquired By: 2007-12-24 15:12:33 Processing Method: 2007-12-24 15:12:12:33 Processing Method: 2007-12-24 15:12:12:12:12:12:12:12:12:12:12:12:12:12:	Sample Name: VSE7 Sample Name: VSE7 Sample Type: 62 hjection #: 1 Run Time: 12.01 Run Time: 145 3.00 1.00 1.00 0.50 0.50	SAMPLE VSE7-A4 Uhtrown 62 50 ul 120 Minutes 145	INFORMATION Acquired By: System Acquired By: System Date Acquired By: System Cate Method Set: 2580, 296 BLSD Date Method Set: 2580, 296 BLSD Processing Method: 2007-12-19 17:42:30 Processing Method: 2007-12-19 15:40:11 Processing Method: 2007-12-19 15:	0 N System 2007-12-19 15:40.01 2007-12-19 17:42:30 2007-254 PDA Max Pot 190.0 - 800.0 PDA Max Pot (190.0 mm to 800.0 mm) PDA Max Pot (190.0 mm to 800.0 mm)
4287-7.4 Peaks - 7.858 Peaks - 7.116 MacO-Mindles A207 A207 A207 A207 A207 A207 A207 A207		Sample Name: Sample Type: Viait hijection #: Painction #: Rinction volume: Rinction Volume: Rinction Volume: Rinction Volume: 2:00- 1:00- 1:00- 1:00- 0:60- 0:60- 0:60-	u Minules	5 ···	40:01 2. 2.00 - 800.0 20.0 mm to 800.0 r
248.2 - 24699 311.7 - 84699 888.7 - 014699	966.51			<del>6628-113699</del>	
		0:00		Peak7 - 6.828 27.144 27.144 27.144	
2.00 4.00 6.00 8.00 1	10,00 12,00 14,00 16,00 18,00	1.00 2.00	3.00 4.00 5.00	6.00 7.00 8.00 9.00 10.00 Minutes	00 11.00 12.00
Peak Results		Peak Results	sults		
Area % Area Height % He		RT Area	% Area Height % He		
4.207 47771 0.11 4997		Peak7 6.828	2.27 109468		
Peak5 5.842 188952 0.44 18803 0.81 Davate 7.116 02400 0.22 0056 0.20		2 7.144 303807 2 Denate 7.374 748028	0.95 49497 1.80		
78767 0.18 8461		Peak9 7.464	0.85 46351		
9.179 699961 1.62 66121		Peak10 7.778	0.11 3694		
10.889 287656 0.67 28029 1.21		6 Peak11 8:293 30487625	95.14 2505891 91.08		
11.242 228610 0.63 20647 0.89					
11.495 58863 0.14 4278 0.19					
12.238 41430817 95.81 2130097 92.22					
17.136 99630 0.23 13461 0.58					
29732 0.07 5738					
-					

Figure S34. HPLC purity checks of compound 7.

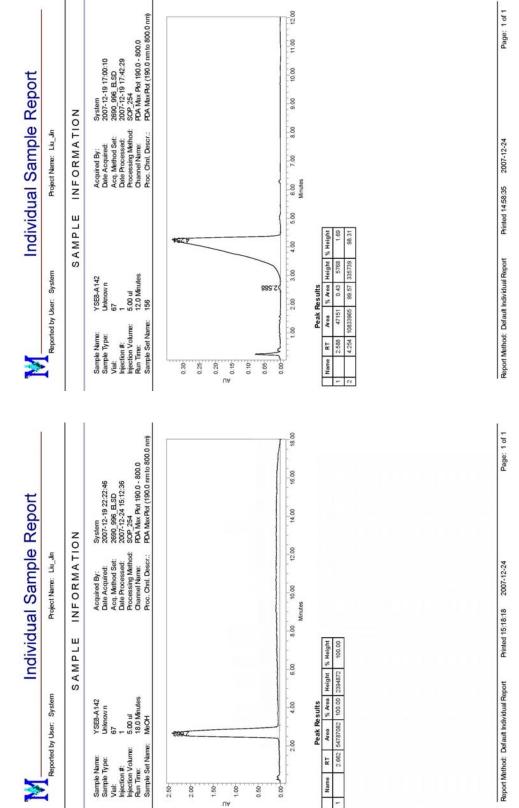


Figure S35. HPLC purity checks of compound 10.

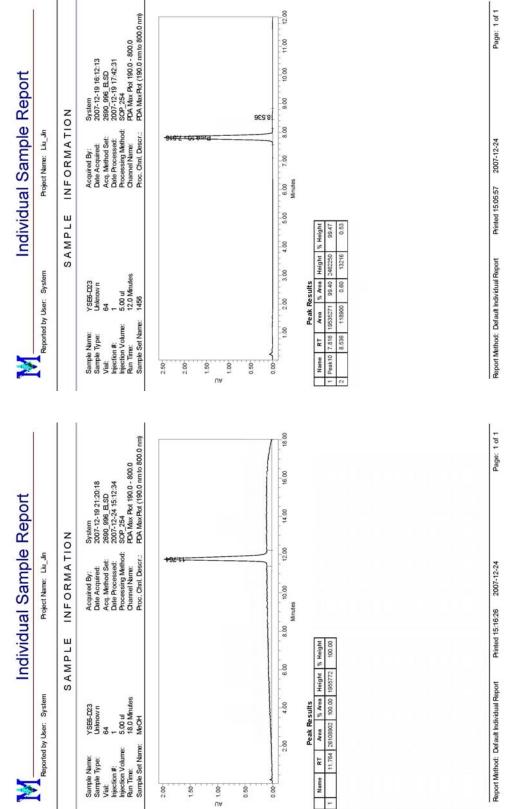


Figure S36. HPLC purity checks of compound 11.

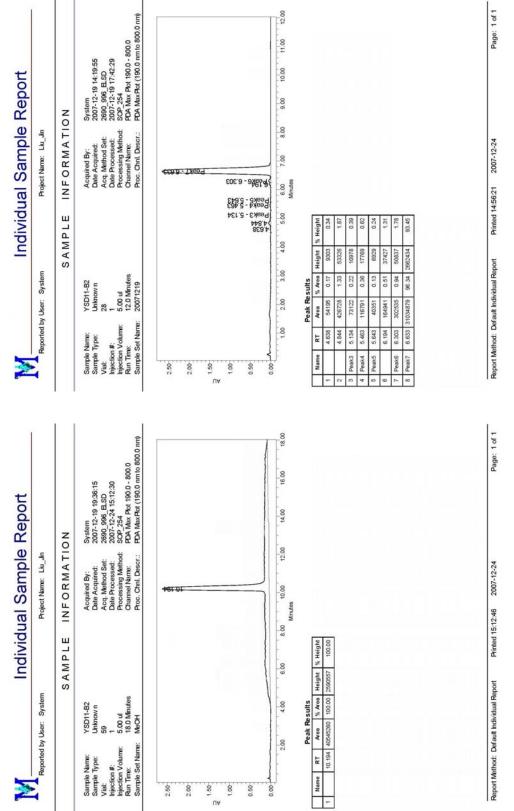


Figure S37. HPLC purity checks of compound 20.

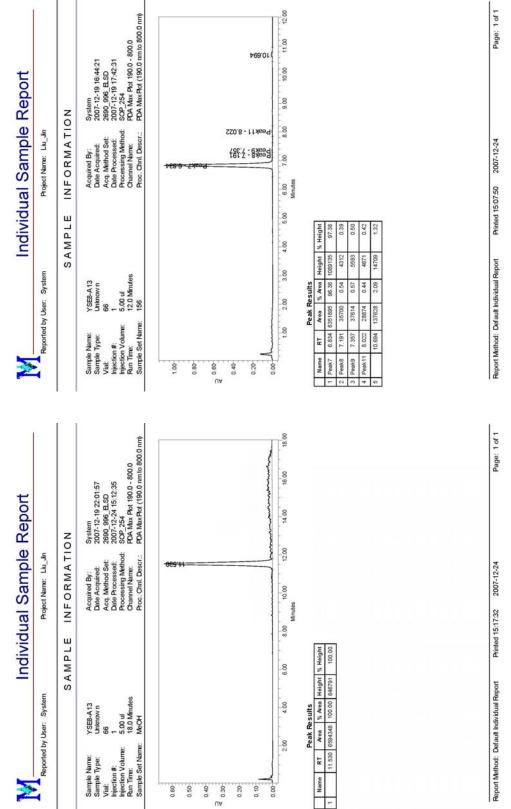


Figure S38. HPLC purity checks of compound 25.

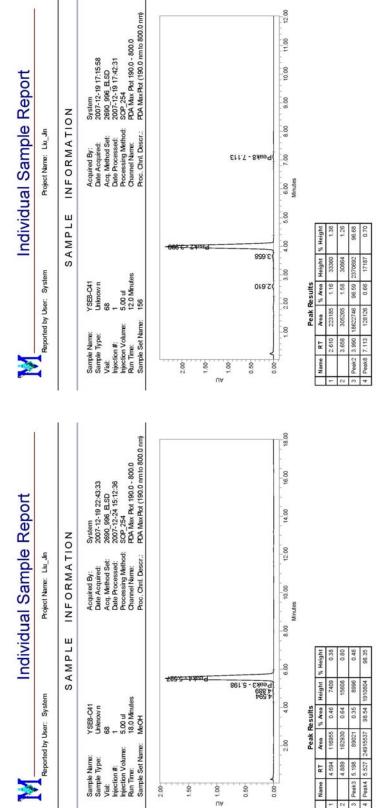


Figure S39. HPLC purity checks of compound 26.

Report Method: Default Individual Report Printed 15:19:22 2007-12-24

Page: 1 of 1

2007-12-24

Printed 15:08:27

Report Method: Default Individual Report

Page: 1 of 1