Bioorganic & Medicinal Chemistry Letters 19 (2009) 5496–5499

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of gibberellin derivatives with anti-tumor bioactivities

Jingbo Chen [a], Zhuxian Sun [a], Yanli Zhang [b], Xianghui Zeng [a], Chen Qing [b,*], Jianping Liu [a], Liang Li [a], Hongbin Zhang [a,*]

[a] Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, Yunnan 650091, PR China
[b] Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650031, PR China

ARTICLE INFO

Article history:
Received 1 May 2009
Revised 26 June 2009
Accepted 14 July 2009
Available online 22 July 2009

Keywords:
Gibberellins
α,β-Unsaturated ketone
Cytotoxic activities
Diterpenes

ABSTRACT

A series of gibberellin based molecules were designed and synthesized. Gibberellin derivatives bearing two α,β-unsaturated ketone units showed strong anticancer activities in MTT assay towards a number of human cancer cell lines including HT29, A549, HepG2 and MKN28. The most potent gibberellin derivative (compound **10**, IC_{50} = 2.9 μM against HT29) inhibited completely the topoisomerase I activity at 8 μg/mL level.

Tetracyclic diterpenes constitute a large class of natural products isolated from plants, microorganisms and marine source.[1] Many of those compounds possess interesting biological activities, especially the gibberellin family and the *ent*-kaurenoid diterpenes. Representative structures of these two diterpenoid groups, ericalycin B (**1**), oridonin (**2**) and gibberellin GA_3 (**3**), are shown in Figure 1. These two families are closely related, gibberellins are biosynthetically from *ent*-kaurenes.[2] A number of *ent*-kaurene diterpenoids possess strong bioactivities against tumor cell lines[3] while gibberellins have been used as important plant growth regulators.[4] Ericalycin B and oridonin are two *ent*-kaurenoids with strong antitumor bioactivity by preventing NF-κB nuclear translocation and inducing IκBα cleavage.[5] A representative structure unit in both molecules is the α,β-unsaturated ketone, especially in the cyclopentane D ring system. Previous studies had well demonstrated that the α,β-unsaturated carbonyl unit incorporated in a cyclopentane or a γ-lactone system is responsible for anti-neoplastic activities.[6] Although gibberellins constitute a large number of compounds with more than 130 members being isolated, an α,β-unsaturated ketone moiety is rarely presented in gibberellin diterpenes. To the best of our knowledge, there is no report on antitumor gibberellins.[7] We were curious to know if such a system were incorporated into the structure of gibberellin GA_3 (**3**), would the resulting gibberellin derivatives bearing an α,β-unsaturated ketone unit display toxicities towards cancer cell lines? By the way, gibberellin GA_3 is abundant and commercially available in large quantities as a plant growth regulator. A research program aiming to assemble α,β-unsaturated ketone moiety to the tetracyclic ring system of gibberellins, especially the D ring, was thus initiated.

Starting from gibberellin GA_3 (**3**), the methyl ester (**4**) and benzyl ester (**5**) were prepared in high yield[8] as outlined in Scheme 1. Diacetate **6** was also prepared by treatment of alcohol **5** with acetic anhydride. With those three compounds (**4**, **5** and **6**) in hand, we initiated the key allylic oxidation to introduce a hydroxyl group to the D-ring of gibberellins. By treatment of substrates with selenium dioxide in the presence of *tert*-butyl hydroperoxide, allylic alcohols were obtained.[9] Having completed the synthesis of compound **7–9**, our attention was then focused on the oxidation of allylic alcohol **9**. To our disappointment, pyridinium dichromate as well as activated MnO_2 failed to promote the desire transformation. The goal was finally realized by a Swern oxidation and the gibberellin derivatives (**10**, **11** and **12**) that bear desired α,β-unsaturated ketone unit were obtained in good yields (see Scheme 2).[10] Carefully examination of the ^{13}C NMR spectrum of compound **10** and **11**, we noticed an unexpected tertiary carbon NMR signal at 65.9 ppm. This NMR signal is unlikely the resonance of a tertiary carbon bearing a hydroxyl group. We speculated that a chlorine atom rather than a hydroxyl group was attached to the C-13 position. The deduced structure was later confirmed by mass spectra (LRMS and HRMS) and further supported by conversion of compound **10** to C-13 hydroxyl compound **14** in the presence of

* Corresponding authors. Tel.: +86 871 5310307 (C.Q.); tel.: +86 871 5031119; fax: +86 871 5035538 (H.Z.).
E-mail addresses: qingchenhh@yeah.net (C. Qing), zhanghb@ynu.edu.cn, zhanghbyd@gmail.com (H. Zhang).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.090

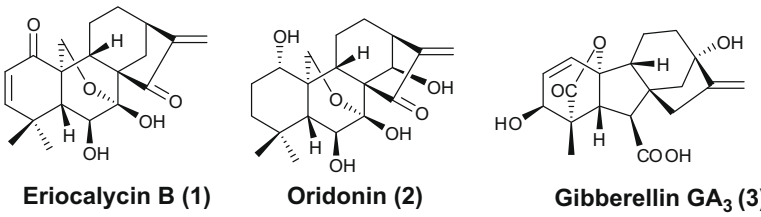


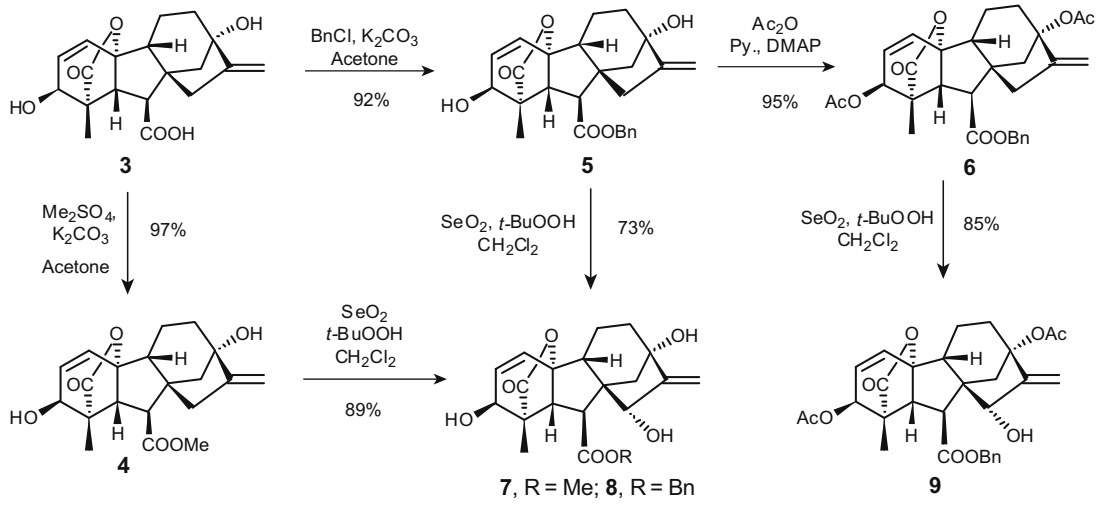
Figure 1. Representative tetracyclic diterpenes (**1–3**).

silver carbonate in wet acetone as indicated in Scheme 3.[11] The chloride compound (**10** and **11**) might be formed by a displacement of the hydroxyl group at C13 position with a chloride anion under the condition of Swern oxidation.[10]

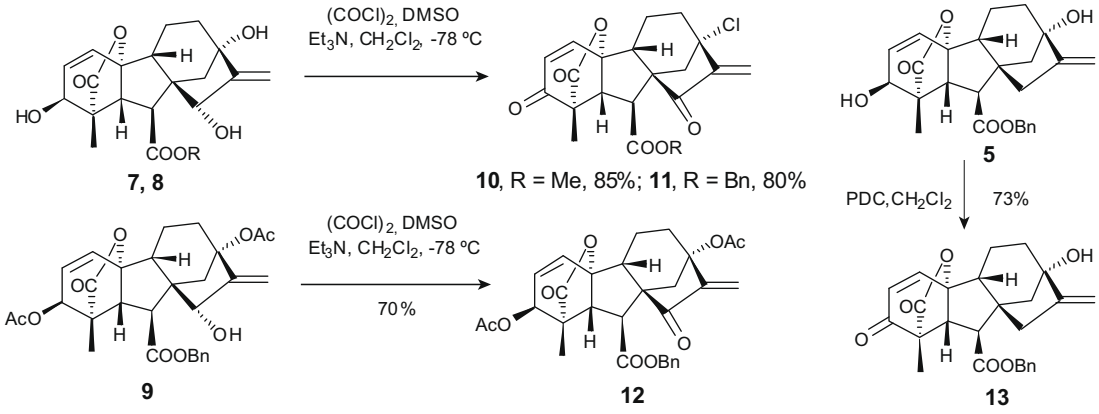
Having obtained gibberellin derivatives bearing a desired α,β-unsaturated ketone moiety, we then initiated a bioassay of these compounds. The cytotoxic potential of all synthesized gibberellin analogues was evaluated in vitro against two human tumor cell lines according to procedures described in the literature.[12] The tumor cell line panel consisted of human promyelocytic leukemia cell line (HL-60) and human bladder carcinoma cell line (BIU-87). Cisplatin (DDP) was used as the reference drug. The results of the cytotoxicity studies were indicated in Table 1 (IC_{50} value, defined as the concentration corresponding to 50% growth inhibition).

To our delight, two compounds (**10**, **11**) with α,β-unsaturated ketone substructure presented in both the D-ring and the A-ring showed strong toxicities toward tumor cells (see Table 1). For compounds without an α,β-unsaturated ketone unit, no cytotoxicities were observed in the bioassay. Only low level of activities was recorded for compounds with one α,β-unsaturated ketone functionality, either in the A-ring (**13**) or the D-ring (**12**) system. These results strongly suggested that both α,β-unsaturated ketone units are required for a better bioactivity.

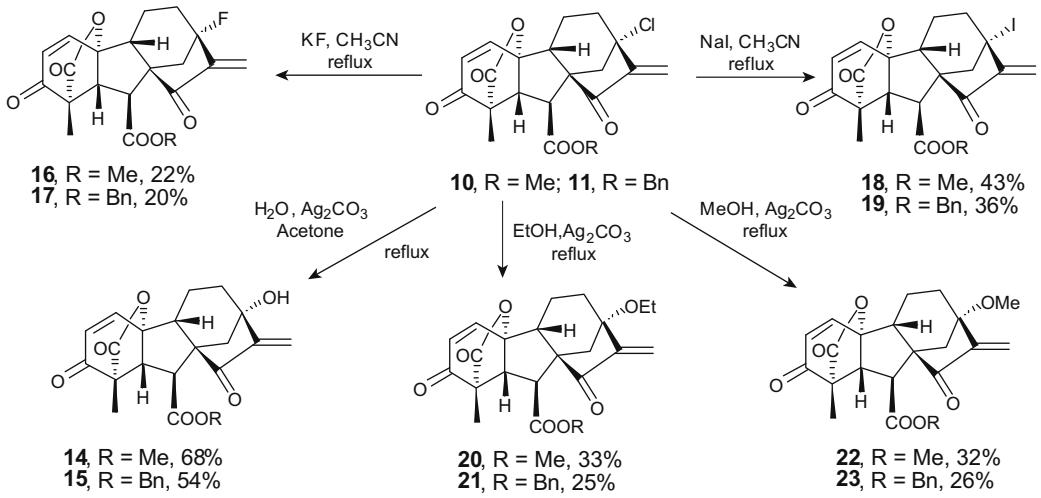
To get further insight toward the structure and activity relationship, a number of C-13 substituted compounds were synthesized by displacement of the chloride at C-13 with corresponding anions (see Scheme 3). Although C-13 florides (**16**, **17**) and C-13 iodides (**18**, **19**) could be obtained in reasonable yields, we failed to



Scheme 1. Synthesis of 15-hydroxyl gibberellin derivatives.



Scheme 2. Synthesis of gibberellin derivatives bearing α,β-unsaturated ketone moiety.



Scheme 3. Synthesis of new gibberellin derivatives bearing α,β-unsaturated ketone moiety.

Table 1
In vitro Cytotoxic activities of gibberellin derivatives (IC_{50}, μg/mL)

Tumor cells	Compounds, IC_{50} (μg/mL)											
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**DDP**
HL-60	>100	>100	>100	>100	>100	>100	>100	6.5	2.7	>40	>40	1.9
BlU-87	>100	>100	>100	>100	>100	>100	>100	1.0	0.5	>40	>40	2.0

Table 2
In vitro cytotoxic activities of gibberellins (IC_{50}, μM)

Tumor cells	Compounds, IC_{50} (μM)													
	10	**11**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**VCR**	**DDP**
HT29	2.9	4.5	65.7	83.3	72.7	59.1	51.9	50.2	34.1	23.3	80.2	48.4	5.3	10.1
A549	60.3	28.8	90.9	23.9	38.9	64.4	53.2	46.0	18.2	24.2	54.5	28.2	2.5	7.6
HepG2	39.8	17.9	37.2	20.5	40.6	44.1	87.0	69.5	120.1	29.6	43.5	35.0	3.7	10.3
MKN28	4.8	6.8	39.6	2.5	4.1	9.8	6.7	3.0	82.7	16.5	3.4	0.8	7.1	4.2

prepare C-13 bromide gibberellins, with a complex mixture being formed. The retaining of configuration at C-13 position suggested that a cation might be generated and quenched with corresponding anions. All these gibberellins were then evaluated for in vitro anti-tumor activity by MTT assay[12] (Table 2) against four human cancer cell lines, namely the human lung adenocarcinoma cell line (A549), human hepatoma cell line (HepG2), human gastric carcinoma cell line (MKN28), human colon carcinoma cell line (HT29). Vincristine (VCR) and cisplatin (DDP) were used as reference compounds. Among all these gibberellin derivatives, compound **10** (IC_{50} = 2.9 μM) showed the highest level of activity followed by **11** (IC_{50} = 4.5 μM) against human colon carcinoma cell lines (HT29), while compound **23** (IC_{50} = 0.8 μM) was found to be most active against MKN28 human gastric carcinoma cell lines (see Table 2).

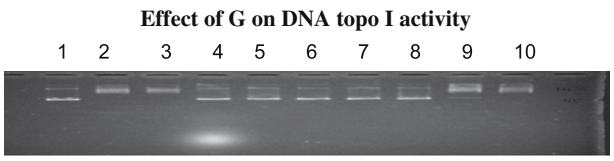


Figure 2. Inhibitory effect of G on DNA topo I activity. Lane 1: 0.25 μg pBR322 DNA only. Lane 2: pBR322 DNA with 2U topo I. Lane 3: Lane 2 with 2% DMSO (solvent). Lane 4: Lane 2 with 125 μg/mL HCPT (hydroxycamptothecin). Lane 5–10: Lane 2 with 1000, 200, 40, 8, 1.6, 0.32 μg/mL compound **11**.

The effects of compound **10**, the most potent compound towards HT29 (IC_{50} = 2.9 μM), on the catalytic activity of topoisomerase I was then measured by the ATP-independent relaxation of supercoiled pBR322 DNA according to procedures described in the literature.[13] The results showed that 2U topo I relaxed fully 0.25 μg supercoiled pBR322 DNA into relaxed form, DMSO did not affected the topo I activity. Compound **10** influenced apparently the activity of topoisomerase I in a dose-dependent manner. The relaxed pBR322 DNA mediated by topo I decreased with increasing **10** concentrations, and supercoiled pBR322 DNA increased. The 8 μg/mL of **10** inhibited completely the topo I activity, resulted in a full conversion of relaxed pBR322 DNA into the supercoiled form (see Fig. 2).

In conclusion, we designed and synthesized a number of gibberellin derivatives bearing α,β-unsaturated ketone units. We also evaluated their anti-tumor activities by in vitro MTT assay. Incorporation of α,β-unsaturated ketone functionality into both the A-ring and D-ring of gibberellins converted the non-cytotoxic molecules to potent anti-tumor agents. Compound **10** showed mild inhibitory effects towards topoisomerase I. Further biological investigation is currently carried out in our laboratory and the results will be reported in due course.

Acknowledgments

This work was supported by grants from the Yunnan Provincial Science & Technology Department (2007B0006Z), the Chinese Ministry of Education for fostering important scientific research program (706052), and the National Natural Science Foudation of China (30660213, 20562013).

References and notes

1. (a) Hanson, J. R. *Nat. Prod. Rep.* **2007**, *24*, 1332; (b) Hanson, J. R. *Nat. Prod. Rep.* **2006**, *23*, 875; (c) Keyzers, R. A.; Northcote, P. T.; Davies-Coleman, M. T. *Nat. Prod. Rep.* **2006**, *23*, 321. and references cited therein.
2. MacMillan, J. *Nat. Prod. Rep.* **1997**, *14*, 221.
3. Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673.
4. Mander, L. N. *Chem. Rev.* **1992**, *92*, 573.
5. (a) Wang, L.; Zhao, W.-L.; Yan, J.-S.; Liu, P.; Sun, H.-P.; Zhou, G.-B.; Weng, Z.-Y.; Wu, W.-L.; Weng, X.-Q.; Sun, X.-J.; Chen, J.; Sun, H.-D.; Chen, S.-J. *Cell Death Differ.* **2007**, *14*, 306; (b) Zhao, Y.; Niu, X.-M.; Qian, L.-P.; Liu, Z.-Y.; Zhao, Q.-S.; Sun, H.-D. *Euro. J. Med. Chem.* **2007**, *42*, 494; (c) Leung, C. H.; Grill, S. P.; Lam, W.; Gao, W.; Sun, H. D.; Cheng, Y. C. *Mol. Pharmacol* **2006**, *70*, 1946; (d) Leung, C. H.; Grill, S. P.; Lam, W.; Han, Q. B.; Sun, H. D.; Cheng, Y. C. *Mol. Pharmacol.* **2005**, *68*, 286.
6. (a) Konaklieva, M. I.; Plotkin, B. J. *Med. Chem.* **2005**, *5*, 73; (b) Umino, K.; Furama, T.; Matzuzawa, N.; Awataguchi, Y.; Ito, Y.; Okuda, T. *J. Antibiot.* **1973**, *26*, 506; (c) Umino, K.; Takeda, N.; Ito, Y.; Okuda, T. *Chem. Pharm. Bull.* **1974**, *22*, 1233.
7. Mander, L. N. *Nat. Prod. Rep.* **2003**, *20*, 49.
8. MacMillan, J.; Willis, C. L. *J. Chem. Soc., Perkin Trans. 1* **1986**, 309.
9. Bhaskar, K. V.; Mander, L. N. *Tetrahedron Lett.* **1996**, *37*, 719.
10. Dolan, S. C.; MacMillan, J. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2741.
11. Compound **10**: colorless crystal, mp: 139–140 °C , ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.18 (1H, d, J = 9.4 Hz), 6.23 (1H, s), 6.07 (1H, d, J = 9.4 Hz), 5.87 (1H, s), 3.63 (3H, s), 3.62 (1H, d, J = 10.2 Hz), 2.82 (1H, d, J = 10.2 Hz), 2.79 (1H, d, J = 11.4 Hz), 2.65 (1H, dd, J = 8.1, 13.6 Hz), 2.50 (1H, d, J = 11.4 Hz), 2.40–2.08 (3H, m), 1.92–1.82 (1H, m), 1.33 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 200.3, 191.1, 172.7, 170.2, 150.7, 145.8, 129.7, 121.4, 89.2, 65.9, 65.4, 61.3, 60.5, 52.5, 48.9, 47.2, 41.6, 39.9, 18.0, 11.9. EIMS m/z (%): 391 [$(M+H)^+$, 4], 390 [$(M)^+$, 4], 361 (16), 360 (43), 358 (100), 326 (31), 303 (16), 286 (42), 267 (43), 251 (50), 236 (52), 222 (40), 217 (23), 213 (15), 195 (13), 165 (29), 152 (20), 141 (22), 128 (40), 115 (50). HRMS m/z. Calcd for $C_{20}H_{19}ClO_6Na$ $(M+Na)^+$: 413.0767. Found: 413.0781. Compound **11**: colorless needles, mp: 177–178 °C, ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.45–7.28 (5H, m), 7.28 (1H, d, J = 9.6 Hz), 6.24 (1H, s), 6.15 (1H, d, J = 9.6 Hz), 5.93 (1H, s), 5.14 (2H, s), 3.72 (1H, d, J = 10.2 Hz), 2.95 (1H, d, J = 10.2 Hz), 2.86 (1H, d, J = 11.4 Hz), 2.72 (1H, dd, J = 7.2, 13.2 Hz), 2.54 (1H, d, J = 11.4 Hz), 2.48–2.12 (3H, m), 2.02–1.92 (1H, m), 1.41 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 200.4, 191.0, 172.7, 169.6, 150.9, 145.8, 134.9, 129.8, 128.7, 128.6, 121.2, 89.2, 67.6, 65.9, 65.5, 61.2, 60.6, 49.1, 47.4, 41.6, 39.8, 18.0, 12.0. EIMS m/z (%): 363 (3), 362 (13), 360 (24), 334 (4), 333 (16), 331 (45), 286 (8), 280 (17), 267 (45), 249 (30), 223 (34), 221 (18), 208 (12), 195 (10), 181 (5), 165 (10), 158 (7), 128 (12), 115 (15), 107 (36), 91 (100). HRMS m/z. Calcd for $C_{26}H_{23}ClO_6Na$ $(M+Na)^+$: 489.1080. Found: 489.1076. Compound **14**: colorless plate, mp: 231–232 °C, ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.20 (1H, d, J = 9.3 Hz), 6.13 (1H, s), 6.08 (1H, d, J = 9.3 Hz), 5.70 (1H, s), 3.67 (1H, d, J = 10.2 Hz), 3.64 (3H, s), 2.83 (1H, d, J = 10.2 Hz), 2.54 (1H, d, J = 10.8 Hz), 2.36–2.18 (3H, m), 2.15 (1H, d, J = 10.8 Hz), 1.98–1.73 (3H, m), 1.35 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 202.4, 191.3, 173.0, 170.6, 152.7, 146.1, 129.7, 118.5, 89.3, 76.0, 65.6, 61.6, 60.9, 52.4, 49.0, 48.1, 40.4, 36.8, 17.6, 12.0. EIMS m/z (%): 373 [$(M+H)^+$, 4], 372 [$(M)^+$, 18], 344 (37), 340 (82), 326 (23), 312 (25), 294 (15), 285 (23), 268 (100), 267 (52), 251 (28), 239 (55), 218 (81), 213 (43), 195 (26), 185 (24), 171 (24), 157 (28), 141 (65), 128 (70), 115(87), 109 (25), 91 (23). HRMS m/z. Calcd for $C_{20}H_{20}O_7Na$ $(M+Na)^+$: 395.1106. Found: 395.1103. Compound **15**: colorless plate, mp: 216–217 °C, ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 7.38–7.16 (6H, m), 6.08 (1H, d, J = 9.6 Hz), 6.06 (1H, s), 5.65 (1H, s), 5.06 (2H, s), 3.67 (1H, d, J = 10.5 Hz), 2.86 (1H, d, J = 10.5 Hz), 2.51 (1H, d, J = 11.1 Hz), 2.32–2.14 (3H, m), 2.07 (1H, d, J = 11.1 Hz), 1.97–1.80 (3H, m), 1.65–1.55 (1H, m), 1.33 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 202.2, 191.3, 173.0, 170.0, 152.9, 146.1, 135.1, 129.7, 128.8, 128.7, 128.7, 118.2, 89.3, 76.0, 67.6, 65.6, 61.6, 60.9, 49.2, 48.2, 40.5, 36.5, 17.6, 12.1. EIMS m/z (%): 448 [$(M)^+$, 2], 420 (3), 357 (3), 342 (18), 313 (8), 295 (5), 280 (16), 267 (21), 249 (7), 221 (16), 213 (4), 195 (5), 171 (4), 151 (13), 148 (20), 128 (12), 115 (12), 107 (26), 91 (100). HRMS m/z. Calcd for $C_{26}H_{24}O_7Na$ $(M+Na)^+$: 471.1419. Found: 471.1408.
12. (a) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589; (b) Zhou, J. J.; Yue, X. F.; Han, J. X.; Yang, W. Y. *Chin. J. Pharm.* **1993**, *24*, 455.
13. (a) Redinbo, M. R.; Stewart, P.; Kuhn, P.; Champoux, J. J.; Hol, W. G. J. *Science* **1998**, *279*, 1504; (b) Redinbo, M. R.; Champoux, J. J.; Hol, W. G. *Biochemistry* **2000**, *39*, 6832.