MedChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

Synthesis and Biological Evaluation of novel derivatives of Gambogenic acid as Anticancer Agent

Peng Huang,[‡a] Zhong Hu,[‡a] Liqin He,*[a] Xiaoshan Wang,[a] Yaxian Wu[a]

Abstract

A series of novel derivatives of gambogenic acid (GNA) were synthesized and evaluated for their *in vitro* antiproliferation activity against four kinds of tumor cell lines. The compounds **3c**, **3f**, **3h** and **3j** displayed potent antiproliferatory activity (IC_{50}: 1.41-2.94 μM on A549 cells，0.98-1.95 μM on HepG2 cells, 0.45-2.86 μM on panc-1 cells and1.13-2.65μM on U251 cells), which are superior to parent compound GNA. **3f** affect significantly the proliferation of tumor cells. Furthermore, **3f** could induce S phase cell cycle arrest and decrease the ratio of Bc1-2/Bax in HepG2 cell. It suggests that **3f** may serve as a potential lead compound for the development of new anticancer drugs.

Keywords: Gambogenic acid; Derivatives; Anticancer activity

[a]*Anhui University of Chinese Medicine, Hefei 230031, PR China*

* Corresponding author. Tel.: +86-551-6516-9387; fax: +86-551-6516-9222; e-mail: hlq661125@126.com

‡ *These two authors contributed equally to this work.*

Introduction

During the past decade, cancer has been the second worldwide killer after heart disease.[1-3] According to GLOBOCAN statistics, cancer accounts for about 56% of the cases and 64% of the deaths in the economically developing world.[4] To date, there is no effective chemotherapy for cancer in humans. However, a lot of researches find Chinese herbs can not only kill tumor cells through multi-channel, but also have little toxic and side effects.[5-8] It is reported that 70% of anticancer drugs were based on compounds derived from natural sources, and the most of which originated from Chinese herbs.[9-11] Therefore, discovery and development of new anti-cancer therapeutic agents from Chinese herb will be very necessary and important.

Gambogenic acid (GNA) is one of the major active ingredients identified from Gamboge. Recently, the researchers have found that the GNA has strong anti-tumor activity, broad anti-tumor spectrum and less toxic. It has been reported that GNA exerts both *in vitro* and *in vivo* inhibition on several tumor cells proliferation，such as HT-29, K562, human lung carcinoma A549 cells, HCC BEL-7402 and CNE-1 cells.[12-16] Moreover, it was not sensitive to the non-tumor cells. However, the further use of GNA was limited due to its poor water-solubility, rapid plasma clearance and vascular stimulation.[17] Therefore, the design and development of more potent and water-soluble derivatives of GNA will be crucial for application of GNA-based anti-cancer agents in clinic.

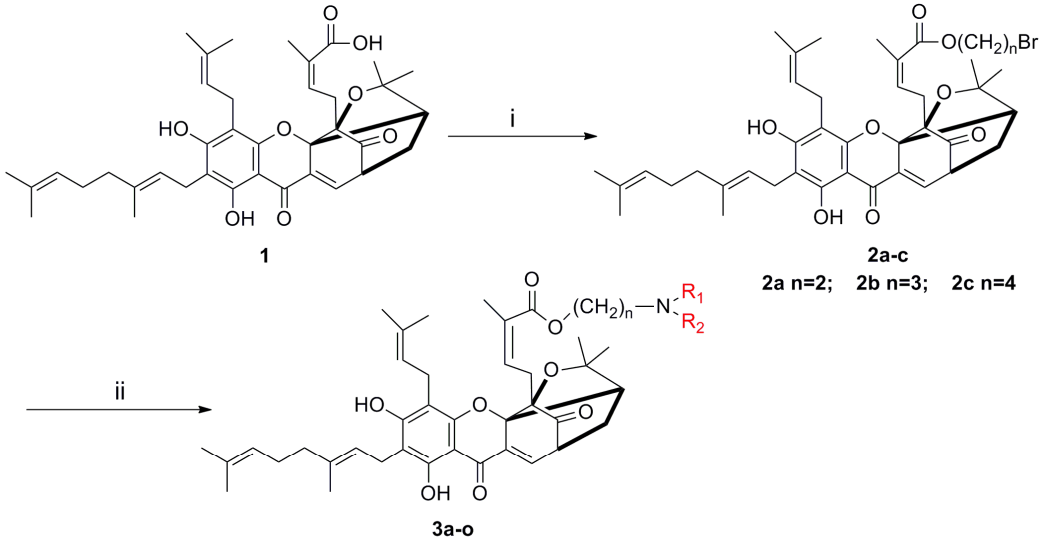
Based on the previous studies of the structure-activity relationships (SARs) of gambogic acid (GA), it was found the 30-carboxy group of GA can tolerate a variety

of modifications with no or little effect on its bioactivity. [18-21] Given that the structure of GNA is similar to that of GA, it is likely that the 29-carboxy group of GNA can also tolerate a variety of modifications. Moreover, it was reported that it improves aqueous solubility and promotes the interaction of between either H-bond donor or acceptor with the intended biological targets by the introduction of amino moiety in the drug molecule. Thus, a series of novel derivatives (**3a-o**) of GNA were designed and synthesized by coupling various hydrophilic alkanolamines to the 29-carboxyl of GNA. On the one hand, it could show new chemical and physical characterization by the combination of GNA and amino unit. Furthermore, it could alter the molecular polarity, solubility, absorption and metabolism, which improve the activity of drug molecules and decrease side-effect. On the other hand, it could promote the anti-cancer activity, particularly for kinase inhibitors by modification of a molecule with amino moiety.[22-27] After unambiguous characterization, these target compounds were evaluated for their inhibitory activity against four kinds of tumor cell lines proliferation. The effects of some potent compounds on tumor cell lines apoptosis were further evaluated. Thus, the paper reports the synthesis and biological evaluation of these derivatives.

Results and discussion

Reference to our previously experiments, GNA was isolated from gamboge and further purified by silica gel column chromatography.[19] The synthesis of compounds **3a–o** is outlined in Scheme 1. GNA was treated with dibromoalkanes bearing two to four carbons in the presence of potassium carbonate in N,N-dimethylformamide

(DMF) to form bromide compounds **2a-c** in yields of 91~93%. The compounds **2a-c** were coupled with various amines in the presence of potassium carbonate in acetonitrile to generate the target compounds **3a–o** in yields of 50~65% after purification by silica gel column chromatography. Meanwhile, the structures of **3a–o** were characterized by IR, MS, ^{1}H NMR, and elemental analysis.[28]



Scheme 1 Synthetic routes of compounds **3a–o,** Reagents and conditions: (i) Br-(CH_2)n-Br (n = 2-4), DMF, K_2CO_3, rt, 2 h; (ii) diethylamine, morpholine, piperidine, N- methyl piperazine and N-hydroxyethyl piperazine, K_2CO_3, CH_3CN, rt, 10-24 h.

The effects of target compounds on the proliferation of human lung carcinoma A549, panc-1, human glioma U251 and human liver hepatocellular carcinoma HepG2 cells *in vitro* were evaluated by MTT assay using GNA as positive control. The IC_{50} values of individual compounds against each tumor cell line are presented in Table 1. In

particular, the compounds **3c**, **3f**, **3h** and **3j** displayed potent inhibition on these cells proliferation superior to GNA. Note that **3f** displayed better anti-proliferative effect than that of **3c**, **3f** and **3j**. For instance, the anti-proliferative effect of compounds **3f** (IC_{50} = 0.45 μM on panc-1 and IC_{50} = 0.98 μM on HepG2 cells) was 9.24- and 4.24-fold more powerful than that of GNA (IC_{50} = 4.16 μM on panc-1 and IC_{50} = 4.16 μM on HepG2 cells), respectively.

Table 1

The cytotoxicity data of GNA and its derivatives [IC_{50} (μM)][a].

Compound	Cell lines			
	A549	**HepG2**	**panc-1**	**U251**
3a	1.61	**2.83**	2.61	3.45
3b	4.27	6.36	1.89	4.93
3c	**1.41**	**1.95**	**2.86**	**2.65**
3d	1.55	0.97	1.89	4.93
3e	2.44	3.87	2.51	3.82
3f	**1.65**	**0.98**	**0.45**	**1.13**
3g	1.30	5.79	2.36	3.31
3h	**2.94**	**2.64**	**2.85**	**2.05**
3i	2.54	3.59	4.66	8.87
3j	**1.51**	**2.75**	**2.52**	**1.18**
3k	3.23	10.84	7.57	17.51
3l	3.17	14.35	18.58	21.55
3m	3.18	2.41	1.84	4.62
3n	3.43	3.92	4.43	2.78
3o	9.53	0.57	0.79	1.46
GNA	3.49	3.23	4.16	3.22

[a]IC_{50} = compound at a concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean IC_{50} from the dose-response curves of at least three independent experiments.

In addition, to better explore the mechanisms underlying the action of **3f**, HepG2 cells were treated with vehicle alone as controls or with different concentrations (0.3125, 0.625 or 1.25 μM) of **3f** for 72 h and stained with Annexin-V FITC and propidium

iodide (PI). The percentages of apoptotic HepG2 cells were determined by flow cytometry analysis (Figure 1). Treatment with vehicle alone did not cause significantly increased frequency of HepG2 cell apoptosis because the percentages of apoptotic HepG2 cells in the vehicle-treated cells were similar to that of unmanipulated control cells (data not shown). Treatment with a low concentration (0.3125 μM) of **3f** induced higher frequency of HepG2 cell apoptosis than that of controls. Moreover, treatment with higher concentrations of **3f** induced higher frequency of HepG2 cell apoptosis and the cytotoxic effects of **3f** appeared to be dose-dependent.

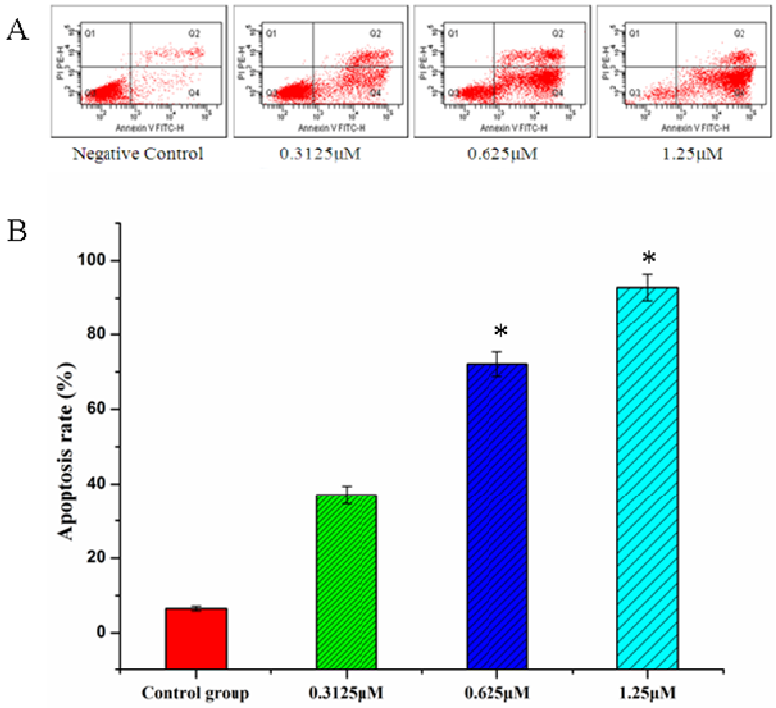


Fig. 1 Compound **3f** induces HepG2 cell apoptosis. HepG2 cells were treated with DMSO (control) or **3f** at various concentrations for 48 h and the frequency of HepG2 apoptosis was determined by annexin V+/PI staining and flow cytometry analysis. A: Flow cytometry analysis of apoptotic HepG2 cells; B: Quantitative analysis; Data are representative charts or expressed as mean ± SEM of the percentage of apoptotic cells from three separate experiments. *p<0.05 *vs*. control cells.

To investigate Bax, Bcl-2 and β-Actin gene expression, we analyzed the gene expression levels

of **3f** treatment after 72 h by Western Blotting. As shown in Fig2, the expression of Bax exhibit more obviously stronger trend than that of control with the concentration of **3f** (0.3125, 0.625 and 1.25 μM) increasing. However, the expression of anti-apoptosis gene Bcl-2 exhibit more obviously decreasing trend than that of control with the concentration of **3f** increasing. The ratio of Bcl-2/Bax might be one of the critical factors of a cell's threshold for undergoing apoptosis, and Bax could bind with Bcl-2 and inhibit its function on suppression of apoptosis.[29-32] Thus, the observed decrease of Bc1-2/Bax illustrated a possible mechanism of the induction of apoptosis. It was shown obviously that compound **3f** could decrease the ratio of Bc1-2/Bax in HepG2 cells. The apoptosis induction of compound **3f** was confirmed in HepG2 cells.

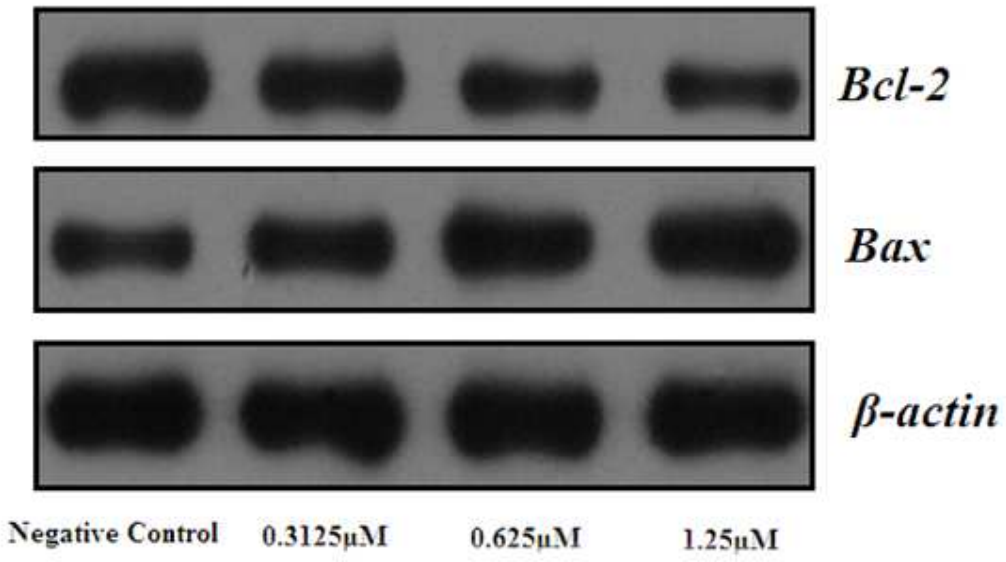


Fig. 2 Effects of **3f** on the expression of Bcl-2, Bax and β-Actin proteins. HepG2 cells were treated with 0.3125 μM, 0.625 μM, and 1.25 μM compound **3f** for 72 h by Western Blotting. The ratio of Bcl-2/Bax was 1.000 (control), 0.598 (0.3125 μM), 0.478(0.625 μM) and 0.251 (1.25 μM).

As shown in Fig3, compound **3f** induced HepG2 cell apoptosis was quantitatively analyze by PI staining assay. The results implied the effect of **3f** treatment within

concomitant time and concentration-dependent manner apoptosis in HepG2 cells. Apoptosis rate was significantly higher after **3f** treatment than control. Moreover, we detected **3f** induced apoptosis whether or not effect on cell cycle distribution. PI staining results focused on **3f** induced S phase cell cycle arrest in HepG2 cells. It is noteworthy that when HepG2 cells were exposed at high concentration S block appeared significantly compared with the low concentration. The results demonstrated that **3f** induced apoptosis might correlate with cell cycle arrest.

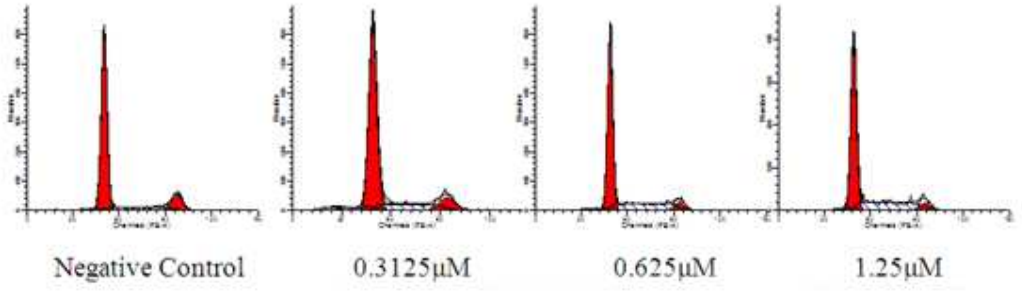


Fig. 3 Effects of Compound **3f** on cycle of HepG2. HepG2 cells were exposed various concentrations (0.3125-1.25 μM) of GNA in 24 h, and the cells were harvested and flow cytometry analyzed for cell cycle distribution by propidium iodide (PI) staining. B. Distribution the various phases of the cell cycle in HepG2 cells. The experiment was repeated twice.

Analysis of SAR revealed that both the length of the aminoalkoxy group and the cyclic amines in GNA derivatives were crucial for their anticancer activity in vitro. Firstly, variation in the length of aminoalkoxy chain affected significantly the in vitro anticancer activity of these derivatives. For example, the compounds **3f**, **3h** and **3j** with a three-carbon alkanol displayed stronger anticancer activity than compounds with a two- and four-carbon linker. It may be that the compounds with a three-carbon aminoalkoxy bind relatively well to the receptor. Secondly, the morpholine derivatives **3b, 3g** and **3l** were less active while the compounds **3a** and **3f** with a N-methylpiperazinyl showed much stronger anticancer activity. Actually, the compound

3f containing a three-carbon alkanol linker and a N- methyl piperazine moiety displayed the strongest anticancer activity than the other derivatives. We speculate that its better anticancer activity was concerned with the suitable polarity of the compounds with N- methyl piperazine. However, the precise SAR of these derivatives need to be further investigated.

Conclusions

In summary, a series of novel GNA derivatives were synthesized by coupling various alkanolamines to the 29-carboxyl group of GNA. All the target compounds displayed inhibition of human tumor cell proliferation. Specially, the compound **3f** appeared to have more strongger inhibitory activity against human tumor cells *in vitro*. Furthermore, **3f** induced high frequency of HepG2 cell apoptosis. The results may provide a framework for the design of new GNA derivatives for the intervention of human tumors in the clinic.

Acknowledgements

This study was supported by a grant from the Nature and Science Foundation of Department of Education, Anhui province in China (No. KJ2013A168, KJ2014A134).

Notes and references

1. E. T. Hawk, E. B. Habermann, J. G. Ford, J. A. Wenzel, J. R. Brahmer, M. S. Chen, L. A. Jones, T. C. Hurd, L. M. Rogers, L. H. Nguyen, J. S. Ahluwalia, M. Fouad, S. M. Vickers, *Cancer*., 2014, **120**,1113-1121
2. R. Siegel, D. Naishadham, A. Jemal, *CA Cancer.J. Clin*., 2012, **62**, 10-29.
3. F. Danhier, O. Feron, V. Preat, *J. Control. Release*., 2010,**148**, 135-146.
4. http://globocan.iarc.fr
5. S. P. Wang, X. Wu, M. Tan, J. Gong, W. Tan, B. L. Bian, M. W. Chen, Y. T. Wang, *J. Ethnopharmacol*., 2012,**140**,33-45
6. N. G. Li, Q. D. You, X. F. Huang, J. X. Wang, Q. L. Guo, X. G. Chen, Y. Li, H. Y. Li, Chin. *J.*

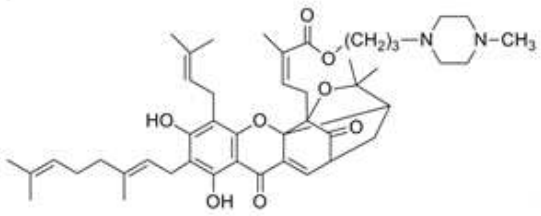
Nat. Med., 2008, 6, 37.
7. J. Asano, K. Chiba, M. Tada, T. Yoshii, *Phytochemistry*., 1996, **41**, 815
8. Roberti, M.; Pizzirani, D.; Simoni, D.; Rondanin, R.; Baruchello, R.; Bonora, C.; Buscemi, F.; Grimaudo, S.; Tolomeo, M. *J. Med. Chem.* 2003, **46**, 3546-3554
9. M. Li-Weber, *Cancer letters*., 2013, **332**, 304-312.
10. Y. Zhou, X. Liu, J. Yang, Q. B. Han, J. Z. Song, S. L. Li, C. F. Qiao, L. S. Ding, H. X. Xua, *Anal. Chim. Acta*., 2008, **629**, 104-118
11. Q. Guo, Q. D. You, Z. Q. Wu, S. T. Yuan, L. Zhao, *Acata Pharmacologica Sinica*., 2004, **25**,769
12. F. G. Yan, Q. L. Li, *Chinese. Pharmacological. Bulletin*., 2011, **27**, 355-359.
13.H. Y. Wu, L. Z. Xia, X. X. Li, H. C. Chang, Q. L. Li, *Environ. Toxicol. Phar*., 2012, **33**,181-190.
14. L. Yang, M. Wang, H. Cheng, Q. L. Li, *China Journal of Chinese Materia Medic*., 2011, **36**, 1217- 1221.
15. Q. L. Li, H. Cheng, G. Q. Zhu, *Biol. Pharm. Bull.*, 2010, **33**, 415-420.
16. C. Peng, J. J. Su, H. Cheng, Q. L. Li, *Journal of Anhui TCM College.*, 2012, **31**, 44-47.
17. X. Huang, Y. J. Chen, D. Y. Peng, Q. L. Li, X. S. Wang, D. L. Wang, W. D. Chen, *Colloids and Surfaces B: Biointerfaces*., 2013,**102**,391-397.
18. S. J. Tao, S. H. Guan, W. Wang, Z. Q. Lu, G. T. Chen, N. Sha, Q. X. Yue, X. Liu, D. A. Guo, *J. Nat. Prod.*, 2008, **72**(1): 117-124.
19. He, L. Q.; Ling, Y.; Fu, L.; Yin, D. K.; Wang, X. S.; Zhang, Y. H . *Bioorg. Med. Chem. Lett.* 2012, **22**(1): 289–292.
20. H. Z. Zhang, S. Kasibhatla, Y. Wang, J. Herich, J. Guastella, B. Tseng, J. Drewe, S. X. Cai, *Bioorg. Med. Chem. Lett*., 2004,**12**, 309–317.
21. J. Kuemmerle, S. C. Jiang, B. Tseng, S. Kasibhatla, J. Drewe, S. X. Cai, *Bioorg. Med. Chem. Lett*., 2008, **16**, 4233–4241.
22. A. K. Ghose, T. Herbertz, J. M. Salvino, J. P. Mallamo, *Drug Discov Today.*, 2006, **11**, 1107.
23. T. J. Ritchie, S. J. F. Macdonald, R. J. Young, S. D. Pickett, *Drug Discov Today.*, 2011, **16**, 164.
24. I. Akritopoulou-Zanze, P. J. Hajduk, *Drug Discov Today.*, 2009, **14**, 291.
25. L. Qiao, S. Choi, A. Case, T. G. Gainer, K. Seyb, M. A. Glicksman, D. C. Lo, R. L. Stein, G. D. Cuny, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6122-6126.
26. S. Cogoi, A. E. Shchekotikhin, A. Membrino, Y. B. Sinkevich, L. E. Xodo, *J. Med. Chem.*, 2013, **56**, 2764.
27. Y. Ling, Z. Q. Wang, H. Y. Zhu, X. M. Wang, W. Zhang, X.Y. Wang, L. Chen, Z. J. Huang, Y. H. Zhang, *Bioorg. Med. Chem.*, 2014, **22**, 374-380
28.General procedure for the synthesis of the target compounds 3a–o: A solution of GNA (1.0 mmol), dibromoalkanes (3.0 mmol) and potassium carbonate (2.0 mmol) in DMF (15 mL) was stirred at room temperature for 1 h. Subsequently, the mixture was poured into H_2O (150 mL) and the resulting products were extracted with EtOAc (50 mL×3). The collected EtOAc layers were washed sequentially with water and saturated NaCl solution, dried over sodium sulfate, and concentrated in vacuo to obtain oil-like materials, which was subsequently purified by column chromatography using (PE/EtOAc = 4:1) to give pure 2a–c in 91–93% yields. A mixture solution of 2a-c (1.0 mmol), K_2CO_3 (2.0 mmol) and appropriate amines (1.5 mmol) in

acetonitrile (15 mL) was stirred at room temperature for 12-26 h. Filtration and removal of the solvent in vacuo afforded the crude product, which was subsequently purified by column chromatography using (CH_3OH/EtOAc = 1:30) to give pure 3a-o in 50–65% yields.

Analytical data for selected final compounds 3f: yellow wax, yield: 57 %; IR (KBr, cm^{-1}): 3422, 2929, 2856, 1708, 1637,1258, 1156. ESI-MS: *m/z* 771 $[M+H]^+$. ^1H NMR ($CDCl_3$, 500 Hz, δ): 12.86(1H, s, 6-OH), 7.47 (d, 1H, *J* = 6.7 Hz), 5.86 (t, 1H, *J* = 6.6 Hz), 5.33 - 5.09 (m, 3H),4.21 - 3.36 (m, 2 H), 3.47-3.18 (m, 4H), 3.18-2.88 (m, 3H), 2.79-2.02 (m, 13H), 2.00- 1.85 (m, 3H), 1.75 (s, 3H), 1.71 (s, 3H), 1.67 (s, 3H), 1.64(s, 9H), 1.56 (s, 3H), 1.39 (m, 4H), 1.28 (s, 3H). Anal. Calcd. for $C_{46}H_{62}N_2O_8$: C, 71.66; H, 8.11; N, 3.63. Found: C, 71.59; H, 8.24; N, 3.51.

29. L. Zhao, Q. L. Guo, Q. D. You, Z. Q. Wu, H. Y. Gu, *Biol. Pharm. Bull.* 2004, **27**, 998-1003
30. S, J. Korsmeyer, J, R. Shutter, D. J. Veis, *Semin. cancer. biol.*, 1993, **4**(6): 327-332.
31. J. C. Reed, *Cell. Death. Differ.*, 2006, **13**, 1378-1386.
32. J. M. Adams, S. Cory, *Science*, 1998, **281**, 1322-1326.

Graphical Abstract



3f

cells	IC_{50} (μM)
A549	1.65
HepG2	0.98
panc-1	0.45
U251	1.13

A series of novel derivatives of gambogenic acid (GNA) were synthesized and evaluated for their *in vitro* antiproliferation activity against four kinds of tumor cell lines. These compounds displayed well potent antiproliferatory activity. Specially, compound **3f** exhibit better inhibition of these tumor cell lines proliferation superior to GNA.