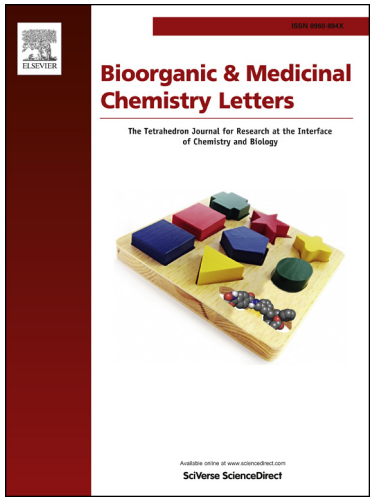


Synthesis and Anti-proliferative Activity of Novel Azazerumbone Conjugates with Chalcones

Vuong Van Truong, Tran Duy Nam, Truong Ngoc Hung, Nguyen Thi Nga, Pham Minh Quan, Luu Van Chinh, Sang-Hun Jung

PII: S0960-894X(15)30103-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.09.069
Reference: BMCL 23150

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 September 2015
Revised Date: 25 September 2015
Accepted Date: 29 September 2015

Please cite this article as: Truong, V.V., Nam, T.D., Hung, T.N., Nga, N.T., Quan, P.M., Chinh, L.V., Jung, S-H., Synthesis and Anti-proliferative Activity of Novel Azazerumbone Conjugates with Chalcones, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.09.069

Synthesis and Anti-proliferative Activity of Novel Azazerumbone Conjugates with Chalcones

Vuong Van Truong[a], Tran Duy Nam[a], Truong Ngoc Hung[b], Nguyen Thi Nga[b], Pham Minh Quan[b], Luu Van Chinh[b*] and Sang-Hun Jung[c*]

[a]*Vietnam – Russia Tropical Centre, 2-Nguyen Van Huyen-Cau Giay-Hanoi-Vietnam*

[b]*Institute of Natural Products Chemistry, Vietnam academy of Science and Technology, 18-Hoang Quoc Viet-Cau Giay-Hanoi-Vietnam. Email: chinhluuvan@gmail.com*

[c]*College of Pharmacy, Chungnam National University, Daejeon, Korea. jungshh@cnu.ac.kr*

Abstract

The conjugation of azazerumbone ((3*E*,7*E*,11*E*)-5,5,8,12-tetramethylazacyclododeca-3,7,11-trien-2-one (**7**)) and 2,4-dihydroxychalcones was carried out for the preparation of novel target compounds **9a-g** with 1-ethylene-4-methylene-1,2,3-triazole linker and **10a-f** with propylene linker between amide nitrogen of azazerumbone and 4-hydroxy group of chalcone. The anti-proliferative activity of these compounds against the LU-1, Hep-G2, MCF-7 and SW480 human cancer cell lines were significantly improved compared to those of azazerumbone or zerumbone. The anti-proliferative activities of (*3E,7E,11E*)-1-((1-(2-(3-hydroxy-4-((*E*)-3-(3-methoxyphenyl)acryloyl)phenoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-5,5,8,12-tetramethyl azacyclododeca-3,7,11-trien-2-one (**9b**) and (*3E,7E,11E*)-1-(3-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenoxy)propyl)-5,5,8,12-tetramethylazacyclododeca-3,7,11-trien-2-one (**10d**) are nearly comparable to those of ellipticine.

Keywords: Zerumbone, Azazerumbone, Chalcone, Conjugate, Anti-proliferative Activity.

*Corresponding author: jungshh@cnu.ac.kr. Tel.; +82 42 821 5939; Fax +82 42 823 6566;

Present address: College of Pharmacy and Institute of Drug Research and development, Chungnam National University, Daejeon 305-764, Korea

A number of natural products[1-5] (Figure 1) has recently recognized to show anticancer activity through inhibition of NF-κB activity. Specially, compounds like curcumin (**1**) [1], zerumbone (**2**)[2] and thiacremonone (**3**)[3,4] from garlic expressed anti-cancer activity through the inhibition of NF-κB. The compound SK-2009 (**4**)[5] (Figure 1) was most potent in suppressing NF-κB activation in KBM-5 leukemic cells. Recently, chalcones such as **5** (figure 1) was reported to have cytotoxic activity against various human cancer cell lines as well as NF-κB inhibitory activity.[6]

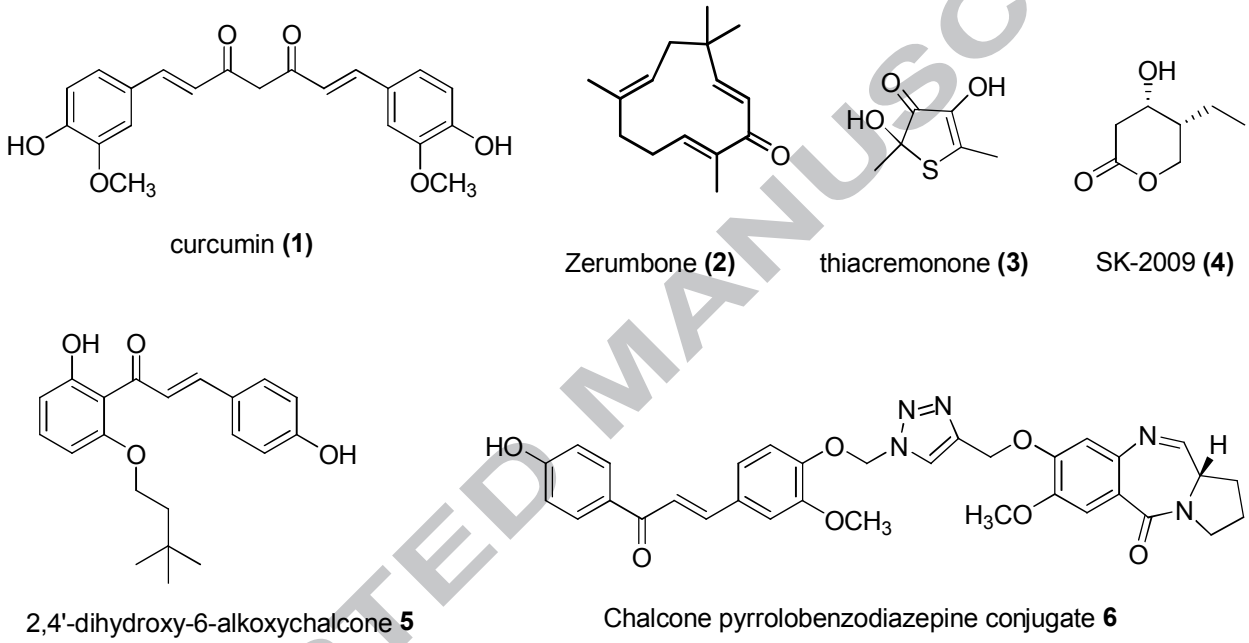


Figure 1: The structures of some compounds exhibiting NF-κB inhibitor activity

Zerumbone[7] (**2**, (2*E*,6*E*,10*E*)-2,6,9,9-tetramethylcycloundeca-2,6,10-trienone, Figure 1) has been focused as a promising cytotoxic sesquiterpene ketone abundantly occurring in the rhizome of *Z. zerumbet*. This unique sesquiterpene is a eleven membered ketone containing pentadienone moiety that is reported to be the center of bioactivity and considered as an active Michael acceptor with preferential activity towards thiol group in certain protein[8]. In 2006, Giang and colleagues[9] studied the inhibition of NF-κB activity by zerumbone and also pointed out that the

α,β-unsaturated ketone group is the key to the activity of this compound. Zerumbone was also reported to inhibit several human cancer cell lines including Hep-G2 (IC_{50} =3.15μg/ml)[10], HeLa (IC_{50}: 2.5 μg/ml)[11, 12] and P-338D1[13]. In these regards, the chemical modifications of zerumbone have attracted significant attention of chemists to find out the suitable structures for application in preventing and treatment of cancer. However, chemical interventions have concentrated on *α,β*-unsaturated ketone moiety led to a significant reduction of anticancer activity [14]

Therefore, we focused to the exploration of novel zerumbone derivatives as an anticancer agent by the conjugation with other bioactive component, while maintaining the bioactive center *α,β*-unsaturated ketone. This molecular conjugation has been known as the rational design of new chemical entities by the fusion of both active compounds and/or pharmacophoric units recognized and derived from known bioactive molecules[15,16] and successfully employed for the enhancement of anti-proliferative activity against various cancer cell lines as shown in **6** (Figure 1) [17,18]. However zerumbone itself does not have proper functional group for conjugation. Thus azazerumbone **7** (Figure 2) was selected for the conjugation as zerumbone motif in this study. Recently, structural transformation of zerumbone to azazerumbone **7** (Figure 2) was performed and these compounds showed antimutagenic activity[19]. Thus zerumbone and azazerumbone are considered to have same range of bioactivities. Azazerumbone **7** has a lactam unit, which can be readily used for conjugation to other functionality. The 2,4-dihydroxychalcones (**8**, Figure 2) were selected as the other part of conjugate designed since chalcones also showed NF-κB inhibitory [6,20] and anti-proliferative activity [6, 21,22]. With these characteristics of azazerumbone and chalcones, conjugated structures **9a-g** with 1-ethylene-4-methylene-1,2,3-triazole linker and **10a-f** with propylene linker between amide nitrogen of azazerumbone and 4-hydroxy group of

chalcone (Figure 2) were designed, prepared and evaluated their anti-proliferative activity against human cancer cell lines.

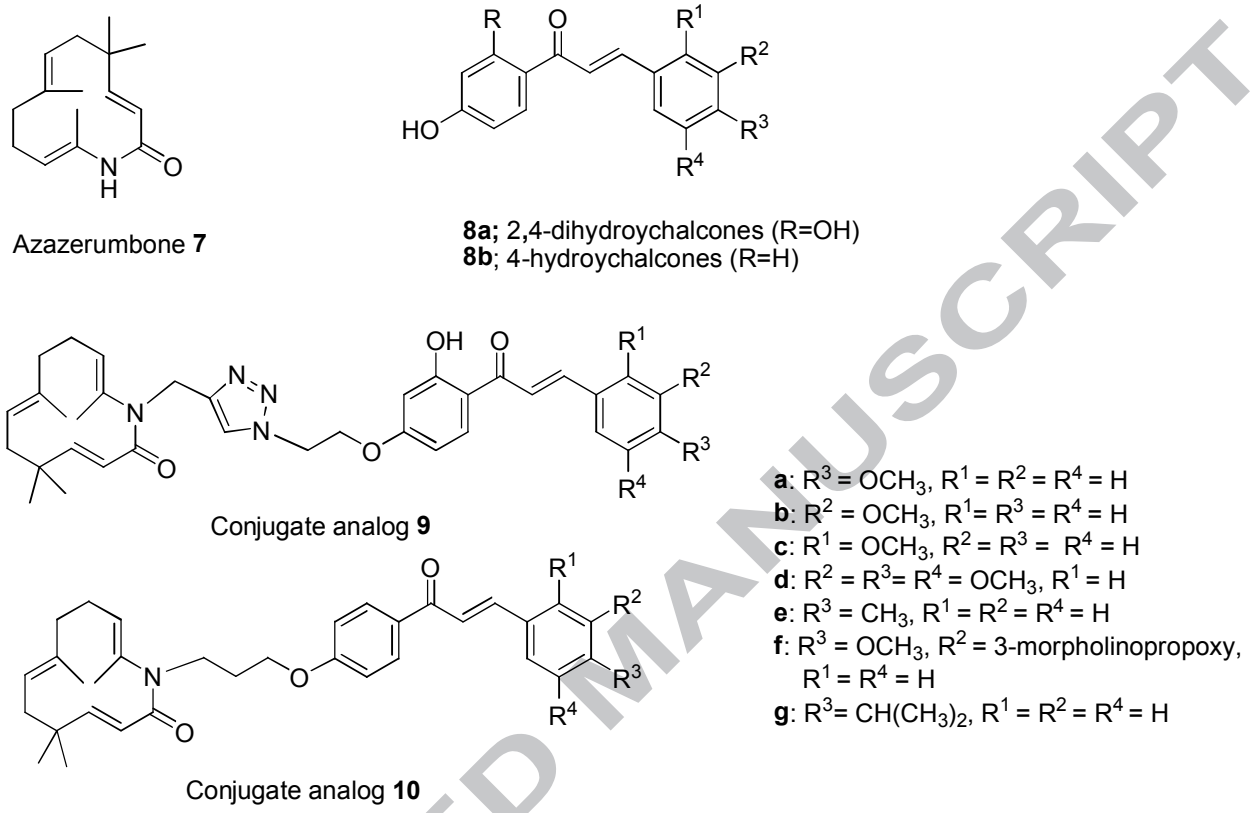
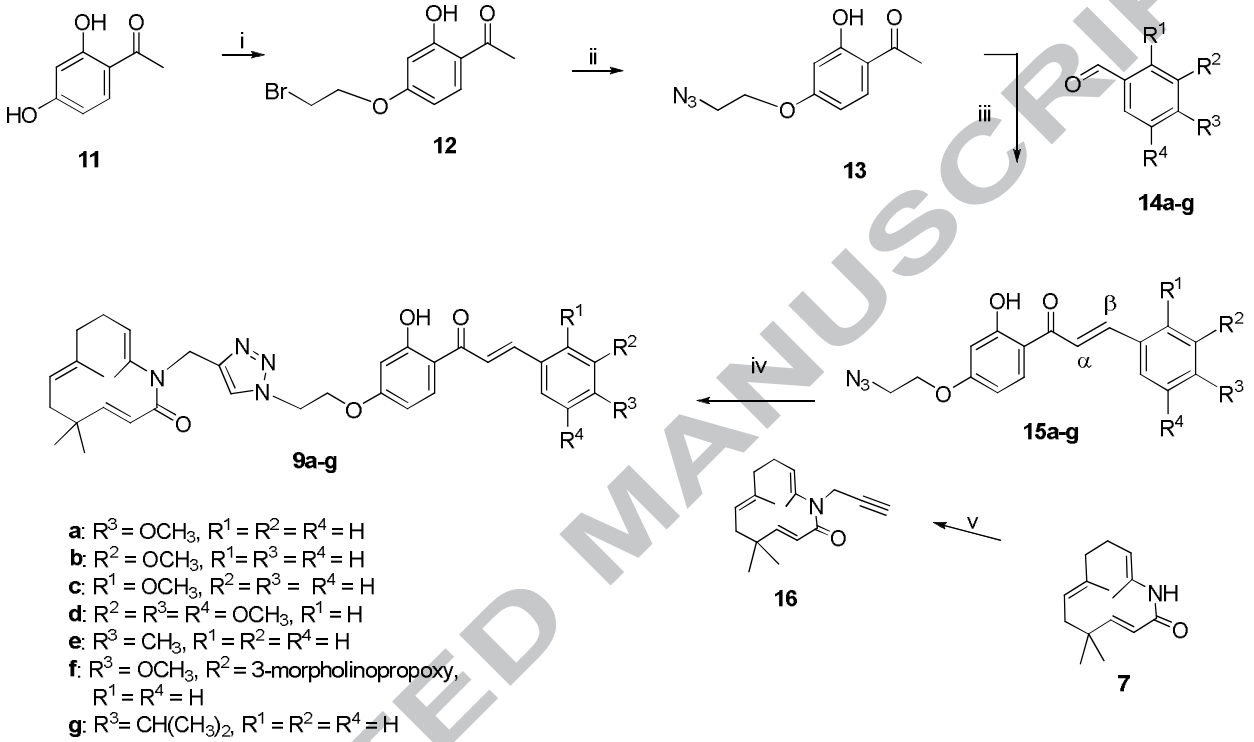


Figure 2. Azazerumbones and new designed conjugates.

The syntheses of designed conjugates **9a-g** and **10a-f** of azazerumbone **7** with chalcones were outlined in Scheme 1 and 2, respectively. For the preparation **9a-g**, azidochalcones **15a-g** were synthesized from 2',4'-dihydroxyacetophenone **11** in three steps (Scheme 1). Firstly, 2',4'-dihydroxyacetophenone **11** was selectively *O*-alkylated by treatment with 1,2-dibromoethane in the presence of K_2CO_3 in acetonitrile at 50 ^oC for overnight to give **12** in 63 % yield. Compound **12** was then reacted with sodium azide in DMSO to afford 4'-(2-azidoethoxy)-2'-hydroxyacetophenone **13** in 90 % yield. Intermediate chalcones **15a-g** were obtained in 81-93 % yields by Claisen-Schmidt condensation in alkaline media of **13** with corresponding aldehydes

14a-g in methanol. Propargylazazerumbone **16** was obtained from the sequential treatment of azazerumbone **7** with sodium hydride and then propargyl bromide. Finally, Click reaction of **16** with corresponding chalcones **15a-g** using CuI as a catalyst in DMSO to give target compounds **9a-g** in 53-59 % yields**.**



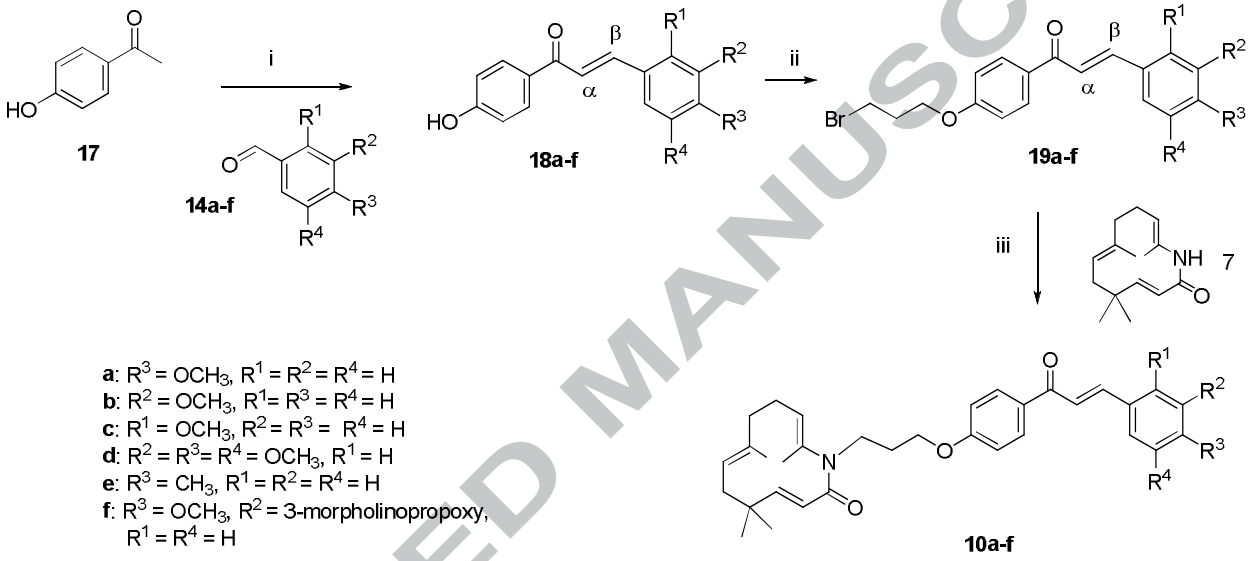
Scheme 1. Preparation of conjugates **9a-g**

Reagents and conditions: (i) 1,2-dibromoethane, MeCN, K_2CO_3, 40 ^oC, 12 h, 63 %; (ii) NaN_3, DMSO, 5 h, rt, 90 %; (iii) KOH, MeOH, rt, 81-93%; (iv) CuI, DMSO, 24 h 53-59%; (v) NaH, propagyl bromide, THF, 0^oC- rt, 87 %.

For the preparation of compounds **10a-f** (Scheme 2), chalcones **18a-f** was obtained in 67-93 % yields from the Claisen-Schmidt reactions of 4'-hydroxyacetophenone **17** with aldehydes **14a-f** [23]. The structure of synthesized chalcones **18a-f** was examined by NMR, MS spectra in good agreement with data given by Hieu[24], Iwata[25], Garg[26] and Ducki[27]. *O*-alkylations of **18a-f** were

performed with 1,3-dibromopropane in the presence of potassium carbonate in acetonitrile at 50 ^{o}C to give 4'-bromopropanoxychalcones **19a-f** in 51-62 % yields. Finally *N*-alkylation reaction of azazerumbone **7** with appropriate 4'-bromopropanoxychalcones **19a-f** in DMF at room temperature using NaH gave target compounds **10a-f** in 58-73 % yields.

The structures of the intermediates and conjugates **9** and **10** are well agreed with IR, MS 1D- and 2D-NMR data [28](Supplementary data).



Scheme 2. Preparation of conjugates **10a-f**

Reagents and conditions: (i) aldehydes **14a-f**, C_2H_5OH, NaOH 10% , rt, 12 h-48 h, 67-93%; (ii) 1,3-dibromopropane, K_2CO_3, 50 ^{o}C, 12 h, 51-62%; (iii) NaH, THF, 0^{o}C- r.t. 58-73%

The evaluation of anti-proliferative activity of the synthesized compounds was performed according to the described protocol[29,30]. The IC_{50} value of the assay was evaluated using five human cancer cell lines: LU, Hep-G2, MCF7, P338 and SW480. The results are listed in Table 1.

Azazerumbone derivatives **9a-g** and **10a-f** exhibited anti-proliferative activity against to five cancer cell lines including LU, Hep-G2, MCF7, 338 and SW480 with IC_{50} values ranging from0.61 to 3.24 μg/mL.

Table 1: The anti-proliferative activity of azazerumbone derivatives **9a-g** and **10a-g**

No	Compounds	IC_{50} (μg/mL) [a]				
		LU[b]	**Hep-G2**[b]	**MCF7**[b]	**P338**[b]	**SW480**[b]
1	**9a**	1.67	2.06	2.85	1.21	3.24
2	**9b**	0.61	1.01	1.12	0.89	0.95
3	**9c**	2.40	1.71	1.17	1.10	0.90
4	**9d**	1.28	1.44	2.78	0.89	2.61
5	**9e**	0.91	0.87	1.21	0.92	1.16
6	**9f**	1.56	1.59	1.13	0.96	0.91
7	**9g**	0.77	0.84	0.93	1.39	0.85
8	**10a**	2.56	1.73	3.17	1.30	2.92
9	**10b**	1.06	1.38	1.37	1.10	1.34
10	**10c**	2.82	1.44	1.56	0.83	1.33
11	**10d**	1.01	0.99	0.58	0.77	0.71
12	**10e**	1.18	1.53	1.21	1.29	1.57
13	**10f**	1.22	1.53	1.07	1.08	0.80
14	**7 (azazerumbone)**	17.82	34.58	15.64	6.25	6.83
15	**Zerumbone**	6.05	13.34	9.67	2.63	3.15
16	**Ellipticine**	0.63	0.36	0.44	0.44	0.38

[a] IC_{50} values shown for these compounds are the average of three determinations.

[b]Cell lines: LU (lung adenocarcinoma, ATCC-HTB-57), Hep-G2 (liver hepatocellular carcinoma, ATCC-HB-8065), MCF7 (breast cancer, ATCC-HTB-22), P338 (leukemia, ATCC-CCl-46) and SW 480 (colon adenocarcinoma, ATCC-CCL-228) cell lines.

The level of activities of **9a-g** and **10a-f** indicated that the azazerumbone conjugates showed approximately two to twenty fold stronger activity against cancer cell lines than those of azazerumbone **7** or zerumbone.

Compounds **9a-g** include the 1-ethylene-4-methylene-1,2,3-triazole linker (Figure 2), which provides more rigidity and space between azazerumbone and chalcone components compared to the flexible propylene linker in **10a**-**f** (Figure2) and therefore the entire structure of **9a**-**f** is likely to be stretched conformation. Although **9a**-**f** exhibited the slightly better activity compared to **10a**-**f**, these significant differences between these linkers did not make any clear discrepancy in anti-proliferative activity in cancer cell lines tested.

For each series, the anti-proliferative activity of the target compounds were varied along with the nature and position of substituents attached to the phenyl moiety of the chalcone components. In series of **9** composed by conjugation of chalcones and azazerumbone with 1-ethylene-4-methylene-1,2,3-triazole linker, the observed anti-proliferative activity indicated that all derivatives **9** showed similar growth inhibition of five human cancer cell lines tested with IC_{50} values ranging from 0.61 to 3.24 μg/mL as shown in Table 1. Among those, **9b** containing methoxy group at position 3 of phenyl in chalcone component showed the best activity against LU cell lines with IC_{50} value of 0.61 μg/mL, followed by the anti-proliferative activity of compound **9g** and **9e** with a bulky isopropyl and a methyl group at position 4 with IC_{50} values of 0.77 and 0.91 μg/mL, respectively. A significant contribution of these groups for anti-

proliferative activity of compounds **9b**, **9e** and **9g** against Hep-G2, MCF7, P388 and SW480 cell lines was also clearly observed.

In series of **10** formed by the conjugation between the chalcones and azazerumbone via propylene linker, the observed anti-proliferative activity indicated that all analogs **10** suppressed the growth of five human cancer cell lines tested with IC_{50} values ranging from 0.58 to 3.17 μg/mL as shown in Table 1. In particular, compound **10d** exhibited good activity against the tested cancer cell lines MCF7, SW480, P338, Hep-G2 and LU with IC_{50} values of 0.58, 0.71, 0.77, 0.99, and 1.01μg/mL, respectively. The simultaneous presence of three methoxy groups at positions 3, 4 and 5 in the phenyl moiety of chalcone component as shown in **10d** seems to be beneficial for anti-proliferative activity. This judge has been further clarified when anti-proliferative activity of compounds **10a, 10b** or **10c** containing a methoxy group at position 2, 3, or 4 has been reduced markedly compared to activity of **10d**.

The anti-proliferative activity of the conjugates **9a-g** and **10a-f** of azazerumbone and 2,4-dihydroxychalcones against the LU-1, Hep-G2, MCF-7 and SW480 human cancer cell lines were significantly improved compared to those of azazerumbone or zerumbone. In series of **9** containing 1-ethylene-4-methylene-1,2,3-triazole linker, a methoxy group at position 3 of phenyl moiety in chalcone component enhances the activity. In series of **10** with propylene linker, the simultaneous presence of three methoxy groups at positions 3, 4 and 5 in the phenyl moiety of chalcone component increases the activity. Finally, the anti-proliferative activities of conjugate derivatives **9b**, **9g** and **10d** are nearly comparable to those of ellipticine as shown in Table 1. Thus, these good anti-proliferative activities of these conjugates of azazerumbone with chalcones obviously implicate good orientation to exploit the pharmacological potential of zerumbone in finding new applications for the treatment of cancer.

Acknowledgment

This work was supported by VAST (VAST04.02/15-16) of Vietnam and Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815).

References and Notes

1. Pan, M. H.; Lin-Shiau, S. Y.; Lin, J. K. *Biochem. Pharmacol.* **2000**, *60*, 1665.
2. Takeda, Y.; Murakami, A.; Aggarwal, B.B. *Oncogene* **2005**, 24, 6975-6969.
3. Ban, J. O.; Yuk, D. Y.; Woo, K. S.; Kim, T. M.; Lee, U. S.; Jeong, H.-S.; Kim, D. J.; Chung, Y. B.; Hwang, B. Y.; Oh, K. W.; Hong, J. T. *J. Pharm. Sci.* **2007**, *104*, 374.
4. Ban, J. O.; Lee, H. S.; Jeong, H. –S.; Song, S.; Hwang, B. Y.; Moon, D. C.; Yoon, D. Y.; Han, S. B.; Hong, J. T. *Mol. Cancer Res.* **2009**, *7*, 870.
5. Anchoori, R. K.; Harikumar, K. B.; Batchu, V. R.; Aggarwal, B. B.; Khan, S. R. *Bioorg. Med. Chem.* **2010**, *18*, 229.
6. Venkateswararao, E.; Sharma, V. K.; Yun, J.; Youngsoo Kim, Y.; Jung, S. H. *Bioorg. Med. Chem.* **2014**, 22, 3386–3392
7. Dung N. X., Chinh T. D., Rang D. D., Leclereq P. A. J. Essent Oil Res., **1993,** 5, 553-555.
8. Xia, Y.; Yang, Z.Y.; Xia, P.; Bastow, K.F.; Nakanishi, Y.; Lee, K.H. *Bioorg, Med. Chem, Lett*. **2000**, 10, 699-701
9. Giang, P. M.; Son, P. T.; Jin, H. Z.; Lee, J. H.; Lee, J. J. *Sci. Pharm*. **2009,** 77, 589–595
10. Abdul, A.B.; Abdelwahad, S.I.; Al-zubairi, A.S.; Elhassan, M.M.; Murali, S.M. *Int. J. Pharm*. **2008**, 4, 301-304

11. Adbul, A. B. H.; Al-Zubairi, A. S.; Tailan, N. D.; Wahab, S.I.A.; Zain, Z.N.M.; Ruslay, S.; Syam, M.M. *Int. J. Pharm.*. **2008**, 4, 160–168.

12. Murakami, A.; Takahashi, D*;* Kinoshita, T.; Koshimizu, K.; Kim H.W.; Yoshihiro, A.; Nakamura, Y.; Jiwajinda, S.; Terao, J.; Ohigashi H. *Carcinogenesis* **2002***,* 23, 795–802.

13. Huang, G.C.; Chien, T.Y.; Chen, LG.; Wang, C.C. *Planta Med*. **2005**, 71, 219-224.

14. Murakami, A.; Tanaka, T.; Lee, J.Y.; Surh, Y. J.; Kim, H. W.; Kawabata, K.; Nakamura, Y.; Jiwajinda, S.; Ohigashi, H. *Int. J. Cancer* **2004**, 110, pp. 481–490.

15. Viegas, Jr. C.; Danuello, A.; Bolzani, V.S.; Barreiro, E.J.; Fraga, C.A.M. *Curr. Med. Chem*. **2007**, 14, 1829.

16. Walsh, J.J.; Bell, A. *Curr. Pharm. Des.* **2009**, 15, 2970.

17. Kamal, A.; Prabhakar, S.; Ramaiah, M. J.; Reddy, P. V.; Reddy, C. R.; Mallareddy, A.; Shankaraiah, N.; Reddy, T.L.N.; Pushpavalli, S.N.C.V.L.; Bhadra, M. P. *Eur. J. Med. Chem.* **2011**, 46, 3820 - 3831.

18. Singh, P.; Raj, R.; Kumar, V.; Mahajan, M. P.; Bedi, P.M.S.; Kaur, T.; Saxena, A. K. *Eur. J. Med. Chem.* **2012**, 47, 594 - 600.

19. Santosh, K.S.C.; Srinivas, P.; Negi, P.S.; Bettadaiah, B.K. *Food Chem.* **2013**, 141, 1097–1103

20. Liu, Y. C.; Hsieh, C. W.; Wu, C. C.; Wung, B. S. *Life Sci.,* **2007**, *80*, 1420-1430.

21. Hieu, B. T.; Thuy, L. T.; Thuy, V. T.; Tien, H. X.; Chinh, L. V.; Hoang, V. D.; Vu, T. K. *Bull. Korean. Chem. Soc.,* **2012,** *33*, 1586-1592

22. Patel, K.; Karthikeyan, C.; Solomon, V.R.; Moorthy, S.H.N.; Lee, H.; Shahu, K.; Deora, G.S.; Trivedi, P. *Lett. Drug. Des. Discov*., **2011**, 8, 308-311

23. Gul H.I., Yerdelen K.O., Das U., Gul M., Pandit B., Li P.K., Mimmock J.R. *Chem. Pharm. Bull*., **2008**, 56, 1657-1681

24. Hieu, B.T.; Thuy, L.T.; Thuy, V.T.; Tien, H.X.; Chinh, L.V.; Hoang, V.D.; Vu, T.K. *Bull. Korean Chem. Soc.*, 2012, 33, 1586-1592.

25. Iwata S., Nishino T., Nagata N., Satomi Y., Nishino H., Shibata S. *Biol. Pharm. Bull*. **1995**, 18, 1710-1713.

26. Garg, N.; Chandra, T.; Archana; Jain A.B.; Kumar A. *Eur. J. Med. Chem*. **2010**, 45, 1529-1535.

27. Ducki, S.; Rennison, D.; Woo, M.; Kendall, A.; Charbert J.F.D.; McGrown, A.T.; Lawrence, N.J. *Bioorg. Med. Chem.* **2009**, 17, 7698-7710.

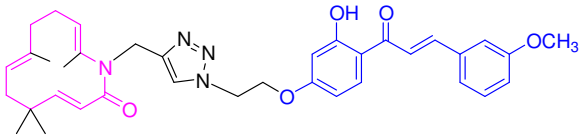
28. The detailed experimental part and spectral data of this article can be found in the Supplementary data.

29. Monks, A.; Scudiero, D.; Skehan, P.; Shoemake, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst*. **1991**, *83*, 757-766.

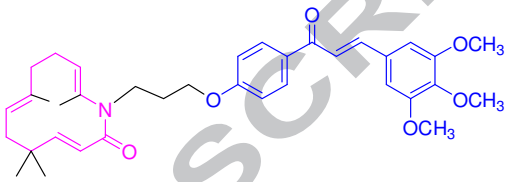
30. Scudiero, D. A.; Shoe-maker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M.J.; Seniff, D.; Boyd, M.R. *Cancer Res*. **1988**, *48*, 4827-4833.

Synthesis and Anti-proliferative Activity of Novel Azazerumbone Conjugates with Chalcones

Vuong Van Truong[a], Tran Duy Nam[a], Truong Ngoc Hung[b], Nguyen Thi Nga[b], Pham Minh Quan[b], Luu Van Chinh[b*] and Sang-Hun Jung[c*]



9b; IC50: 0.61(LU), 1.01(Hep-G2), 1.12(MCF7), 0.89(P388), 0.95(SW480) µg/mL



10d; IC50: 1.01(LU), 0.99(Hep-G2), 0.58(MCF7), 0.77(P388), 0.71(SW480) µg/mL