

六種多酚化合物抑制腫瘤壞死因子- 和核因子- B途徑的對接預測

孫瑞強、李世傑,李桂仁

E-mail: 387168@mail.dyu.edu.tw

摘要

本研究從具有消炎作用的中藥中，選取藥效較強而且常被使用的六種中藥多酚：薑黃素、兒茶素、芥子醇、丁香?、雷公藤甲素和木犀草素，分為兩部分的主題來進行研究；第一部分是以前述六種中藥多酚以Autodock 4的軟體來作和TNF- 的對接預測；發現在當作以抑制TNF- 的消炎藥劑的單方面，用雷公藤甲素或是薑黃素會比較好，這二者的藥效既強又穩定。若用做消炎的複方時，因為TNF- 的口袋是三個，根據研究結果，發現有三種使用方法：(a) 同時使用薑黃素，兒茶素，芥子醇和木犀草素。(b) 同時使用薑黃素，丁香?，芥子醇和木犀草素。(c) 同時使用兒茶素，丁香?，和雷公藤甲素和木犀草素。第二部分是以前述六種中藥多酚當作小分子，胞內的NF- B途徑 (TNFR1、 TRAF2、 TRADD、 RIP1、 IKK) 分別當作大分子，以Autodock 4的軟體來作對接預測；發現木犀草素可以強烈的抑制TRAF2、 TNFR1 和 TRADD三者的功能，木犀草素可以聯結住RIP1，阻斷RIP1訊號的傳遞；木犀草素也和IKK 的C-末端強力結合，阻止IKK 和IKK 以及IKK 形成三聚體，產生功能。也就是木犀草素可作為細胞外的TNF- 的抑制劑，也可以是細胞內NF- B途徑的抑制劑。本研究發現這六種多酚除了可以在細胞外以抑制TNF- ，產生消炎作用以外，其中的木犀草素還可以在細胞內以抑制NF- B途徑，來產生消炎作用。

關鍵詞：薑黃素、兒茶素、芥子醇、丁香?、雷公藤甲素、木犀草素、NF- B途徑

目錄

目錄 封面內頁 簽名頁 中文摘要.....	iii
英文摘要.....	iii
v 誌謝.....	vi
目錄.....	vi
vii 圖目錄.....	ix
表目錄.....	ix
xi 第一章 緒言.....	1
第一節 研究背景與動機.....	1
第二節 研究問題與目的.....	2
第二章 文獻回顧.....	6
第一節 TNF- 和NF- B對於人體的保護及引起的疾病.....	6
第二節 六種多酚：薑黃素、兒茶素、芥子醇、丁香?、雷公藤甲素和木犀草素抑制TNF- 的活性的相關研究.....	13
第三節 木犀草素抑制核因子- B的相關研究.....	17
第四節 SWISS-MODEL Workspace的相關研究.....	18
第五節 Autodock 4 (包含ADT)的相關研究.....	20
第六節 LIGPLOT的相關研究.....	21
第七節 SwissPdb Viewer的相關研究.....	22
第八節 PROCHECK的相關研究.....	22
第九節 VERIFY3D的相關研究.....	23
第十節 ERRAT的相關研究.....	24
第十一節 ChemSpider.....	24
第三章 材料與方法.....	25
第一節 六種多酚對於腫瘤壞死因子- 的對接預測.....	25
第二節 木犀草素抑制核因子- B途徑的對接預測.....	34
第四章 結果與討論.....	44
第一節 六種多酚對於腫瘤壞死因子- 的對接預測的結果.....	44
第二節 木犀草素抑制核因子- B的對接預測的結果.....	60
第五章 結論.....	72
參考文獻.....	76
附錄.....	89
圖目錄 圖目錄 圖3.1 儲存到AutoDock中的1A8MB的DeepView project的pdb檔.....	28
圖3.2 6個多酚的化學結構：(a).薑黃素(b).兒茶素(c).芥子醇(d).丁香?(e).雷公藤甲素(f).木犀草.....	33
圖3.3a 儲存到AutoDock中的 TNFR(41到201) 1extA的 Deep View project的pdb檔.....	38
圖3.3b 儲存到AutoDock中的TNFR(356到442)1ichA的DeepView project的pdb檔.....	39
圖3.4 儲存到AutoDock中的 TRADD(1f2hA)的Deep View project的pdb檔.....	40
圖3.5 儲存到AutoDock中的 TRAF2(1ca9B)的DeepView project的pdb檔.....	41
圖3.6 儲存到AutoDock中的 RIP1 (1qcfA) 的DeepView project的pdb檔.....	42
圖3.7 儲存到AutoDock中的IKK (1cm8B) 的DeepView project的pdb檔.....	43
圖4.1 這六個多酚和TNF- 表面的對接結果.....	52
圖4.2 TNF- 中相同口袋的對接配體群.....	53
圖4.3 配體：薑黃素、兒茶素、芥子醇、丁香?、雷公藤甲素和木犀草素的凡得瓦爾力和氫鍵作用力.....	54
圖4.4 對接結果中的 - 型態原子之間的作用力和 - 陽離子的用力.....	57
圖4.5 對接結果中的 - 型態原子之間的作用力和 - 陽離子的作用力.....	68
表目錄 表3.1 TNF- (1A8MB) 同源模型的模板和品質.....	27
表3.2 在ChemSpider(http://www.chemspider.com/)的小分子的最新發表的資料.....	31
表3.3 模板和同源模型的品質.....	37
表4.1 以AUTODOCK 4.0所計算出的能量能量(以kcal/ mol表示).....	

.....48 表4.2 氫鍵作用力以及每一個氫鍵的能量.....	58 表4.3 凡得瓦爾力.....
... 59 表4.4 AUTODOCK 4.0計算出的能量(kcal/mol).....	67 表4.5 氫鍵相互作用和每一個鍵的能量.....
...69 表4.6 凡得瓦力作用力.....	70 表4.7 疏水性作用力.....
71 附錄 附錄1 名詞對照表.....	89 附錄2 發表論文列表.....
..... 94 附錄3發表論文.....	95 附錄4 SCI發表論文.....
.... 96 附錄5 autodock(介紹).....	97 附錄6 ChemSpider(介紹).....
....100 表目錄 表3.1 TNF – (1A8MB) 同源模型的模板和品質.....	27 表3.2 在ChemSpider(http://www.chemspider.com/)的小分子的 最新發表的資料.....
..... 37 表4.1 以AUTODOCK 4.0所計算出的 能量能量(以kcal/ mol表示).....	31 表3.3 模板和同源模型的品質...
..... 48 表4.2 氫鍵作用力以及每一個氫鍵的能量.....	58 表4.3 凡得瓦爾力.....
..... 59 表4.4 AUTODOCK 4.0計算出的 能量(kcal/mol).....	67 表4.5 氫鍵相互作用和每一個鍵的能量.....
... 69 表4.6 凡得瓦力作用力.....	70 表4.7 疏水性作用力.....
71 附錄 附錄1 名詞對照表.....	89 附錄2 發表論文列表.....
..... 94 附錄3發表論文.....	95 附錄4 SCI發表論文.....
.... 96 附錄5 autodock(介紹).....	97 附錄6 ChemSpider(介紹).....
..... 100	

參考文獻

1. Abdelhamid, L., Judith, S. and Muhammad, Z. 2005. Triptolide suppresses proinflammatory cytokine-induced matrix metalloproteinase and aggrecanase-1 gene expression in chondrocytes. *Biochemical and Biophysical Research Communications* 327,320-327.
2. Aggarwal, B. B. and Shishodia, S. 2004. Suppression of the nuclear factor-kB activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Ann NY Acad Sci* 1030,434 – 441.
3. Agullo, G., Gamet-Payrastré, L., Manenti, S., Viala, C., Remesy, C., Chap, H. and Payrastré, B. 1997. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition, *Biochem. Pharmacol* 53, 1649 – 1657.
4. Ahima, R. S. and J.S. Flier. 2000. " Leptin " , *Annu. Rev. Physiol* 62, 413.
5. Arbibe, L., D. Vial, I. R. Chupin, N. Havet, M., Huerre, B. B., Vargaftig, and L. Tourqui.1997. Endotoxin induces expression of type II phospholipase A2 in macrophages during acute lung injury in guinea pigs. *J. Immunol* 159, 391.
6. Arenzana-Seisdedos, F., Thompson, J., Rodriguez, MS., Bachelier, F., Thomas, D. and Hay, RT. 1995 .Inducible nuclear expression of newly synthesized I kappa B alpha negatively regulates DNA-binding and transcriptional activities of NF-kappa B. *Mol Cell Biol*.15:2689 – 2696.
7. Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoaka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T. and Matsuzawa, Y. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257,79 – 83.
8. Arner, P. 2002. Insulin resistance in type 2 diabetes: role of fatty acids. *Diabetes Metab. Res. Rev* 18 (Suppl. 2), 5 – 9.
9. Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. 2006. The SWISS-MODEL Work space: A web-based environment for protein structure homology modeling. *Bioinformatics* .22, 195-201.
10. Baeuerle, P. A. and Henkel, T. 1994. Function and activation of NF-kB in the immune system. *Annu. Rev. Immunol* 12, 141 – 179.
11. Baldwin, A. S. 1996. The NF- B and I B proteins: new discoveries and insights. *Annu. Rev. Immunol* 14, 649 – 681.
12. Barry, M. A. and Eastman, A. 1993. Identification of deoxyribonuclease II as an endonuclease involved in apoptosis. *Arch. Biochem. Biophys.* 300, 440 – 450.
13. Beutler, B. and Cerami, A. 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* 320, 584 – 588.
14. Bowie, J. U., Luthy, R. and Eisenberg, D. 1991.A method to identify protein sequences that fold into a known three-dimensional structure. *Science* 253,164-170.
15. Bowie, J. U., Eisenberg, D. and Luthy, R. 1992. Assessment of protein models with three-dimensional profiles. *Nature* .365,83-85.
16. Bradley, JR. 2008. TNF-mediated inflammatory disease. *J Pathol* 214: 149 – 160.
17. Brown, NR., Noble, ME., Endicott, J. A. and Johnson, L. N.1999. The structure basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases.*Nat Cell Biol*.1(7):438-43.
18. C. Hubac., J. Ferran., A. Tremolieres. and A. Kondorosi. 1994. Luteolin uptake by *Rhizobium meliloti*: evidence for several steps including an active extrusion process. *Microbiology*. 140,2769-2774.
19. Cabannes, E., Khan, G., Aillet, F., Jarrett, RF. and Hay, RT. 1999. Mutations in the Ikb α gene in Hodgkin's disease suggest a tumour suppressor role for I κ B α . *Oncogene* 18 (20): 3063 – 70.
20. Chan, FK., Siegel, RM. and Lenardo, MJ. 2000. Signaling by the TNF receptor superfamily and T cell homeostasis. *Immunity* 13, 419-422.
21. Chen, ZJ .2005. Ubiquitin signalling in the NF-kappaB pathway. *Nat Cell Biol* 7: 758 – 765.
22. Chen, C.Y., Peng, W. H., Tsai, K. D. and Hsu, S. L. 2007. Luteolin suppresses inflammation-associated gene expression by blocking NF-kappaB and AP-1 activation pathway in mouse alveolar macrophages. *Life. Sci.* 30, 1602 – 1614.
23. Cho, J. Y., Nam, K. H., Kim, A. R., Park, J., Yoo, E. S., Baik, K. U., Yu, Y. H. and Park, M. H. 2001. In-vitro and in-vivo immunomodulatory effects of syringin. *J. Pharm Pharmacol* .53,1287-1294.
24. Choi, YJ., Kim, TG. and Kim, YH. 2003 . Immunosuppressant PG490 (triptolide) induces apoptosis through the activation of caspase-3 and down-regulation of XIAP in U937 cells. *Biochem Pharmacol* .66:273-280.
25. Choi, J., K. M. Shin, H. J. Park, H. J. Jung, H. J. Kim, Y. S. Lee, J. H. Rew, and K. T. Lee. 2004. Anti-inflammatory and antinociceptive effects of sinapyl alcohol and its glucoside syringin. *Planta Med.* 70,1027.
26. Collins, T. and Cybulsky, M. I. 2001. NF- B: pivotal mediator or innocent bystander in atherogenesis? *J. Clin. Invest.* 107,255 – 264.
- 27.

Colovos, C. and Yeates, T. O. 1993. Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci.* 2, 1511-1519. 28.

Cunningham, B. D., Threadgill, M. D., Groundwater, P. W., Dale, I. L. and Hickman, J. A. 1992. Synthesis and biological evaluation of a series of flavones designed as inhibitors of protein tyrosine kinases. *Anticancer Drug Des.* 7, 365 – 384. 29.

De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, DU., Jin, R., Jones, J., Cong, R. and Franzoso, G. 2001. Induction of gadd45beta by NF-kappaB downregulates pro-apoptotic JNK signalling. *Nature*, 414, 308 – 313. 30.

Devin, A., Cook, A., Lin, Y., Rodriguez, Y. and Kelliher, M. 2000. The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* 12:419-429. 31.

Devin, A., Lin, Y., Yamaoka, S., Li, Z. and Karin, M. 2001. The alpha and beta subunits of IkappaB kinase (IKK) mediate TRAF2-dependent IKK recruitment to tumor necrosis factor (TNF) receptor 1 in response to TNF. *Mol Cell Biol.* 21:3986-3994. 32.

Dong, C., Davis, R.J. and Flavell, RA. 2002. MAP kinases in the immune response. *Annu Rev Immunol* 20:55 – 72. 33.

Favelyukis, S., Till, JH., Hubbard, SR. and Miller, WT. 2001. Structure and autoregulation of the insulin-like growth factor 1 receptor kinase. *Nat Struct Biol.* 8(12):1058-63. 34.

Feingold, K. R., Marshall, M., Gulli, R., Moser, A. H. and Grunfeld, C. 1994. Effect of endotoxin and cytokines on lipoprotein lipase activity in mice. *Arteriosclerosis and Thrombosis* 14, 1866 – 1872. 35.

Feingold, K. R., Hardardottir, I. and Grunfeld, C. 1998. Beneficial effects of cytokine induced hyperlipidemia. *Z. Ernahrungswiss*; 37 Suppl. 1, 66 – 74. 36.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S. and Girardin, S. E. 2007. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 beta generation. *Clin. Exp. Immunol.* 147,227 – 235. 37.

Ferriola, P. C., Cody, V. and Middleton, E. Jr. 1989. Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem Pharmacol* 38,1617 – 1624. 38.

Festjens, N., T. Vanden, Berghe, S. Cornelis, and P. Vandenabeele. 2007. RIP1, a kinase on the crossroads of a cell's decision to live or die. *Cell Death Differ.* 14:400 – 410. 39.

Fotsis, T., Pepper, M. S., Aktas, E., Breit, S., Rasku, S., Adlercreutz, H., Wahala, K., Montesano, R. and Schweigerer, L. 1997. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis, *Cancer Res.* 57, 2916 – 2921. 40.

Galluzzi, L., O. Kepp, and G. Kroemer. 2009b. RIP kinases initiate programmed necrosis. *J. Mol. Cell. Biol.* 1:8 – 10. 41.

Ghosh, S., May, M. J. and Kopp, E. B. 1998. NF- κ B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16,225 – 260. 42.

Gilmore, T. D. 2006. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene* 25, 6680 – 6684. 43.

Graziani, Y., Erikson, E. and Erikson, R. L. 1983. The effect of quercetin on the phosphorylation of the Rous sarcoma virus transforming gene product in vitro and in vivo. *Eur J Biochem* 135,583 – 589. 44.

Grunfeld, C. and Feingold, K. R. 1991. Tumour necrosis factor, cytokines and the hyperlipidemia of infection. *Trends Endocrinol Metab.* 2, 213 – 219. 45.

Guex, N. and Peitsch, M. C. 1997. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* .18, 2714-2723. 46.

Harborne, J. B. and Williams, C. A. 2000. Advances in flavonoid research since 1992. *Phytochemistry* .55,481 – 504. 47.

Hayden, M. S. and Ghosh, S. 2008. Shared principles in NF-kappaB signaling. *Cell* 132,344 – 362. 48.

Hikim, A. P. S., Lue, Y. H., Wang, C., Reutrakul, V., Sangsuwan, R., Swerdloff, R. S., 2000. Post-testicular antifertility action of triptolide in the male rat: evidence for severe impairment of caudal epididymal sperm ultrastructure. *J. Androl.* 21 (3), 431 – 437. 49.

Hsu, H., Xiong, J. and Goeddel, DV .1995. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* .81,495-504. 50.

Huang, Y. T., Hwang, J. J., Lee, P. P., Ke, F. C., Huang, J. H., Huang, C. J., Kandaswami, C., Middleton, E. Jr. and Lee, M. T. 1999. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. *Br. J. Pharmacol.* 128, 999 – 1010. 51.

Huey, R., Morris, G. M., Olson, A. J. and Goodsell, D. S. 2007. A Semiempirical Free Energy Force Field with Charge-Based Desolvation. *J Comput Chem* 28, 1145-1152. 52.

Hymowitz, SG., Christinger, HW., Fuh, G., Ultsch, M., O'Connell, M., Kelley, RF., Ashkenazi, A. and de Vos, AM. 1999. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol Cell* 4(4):563-71. 53.

Izumi, KM., Cahir-McFarland, ED., Ting, AT., Riley, EA. and Seed, B. 1999. The Epstein-Barr virus oncoprotein latent membrane protein 1 engages the tumor necrosis factor receptor-associated proteins TRADD and receptor-interacting protein (RIP) but does not induce apoptosis or require RIP for NF-kappaB activation. *Mol Cell Biol* 19, 5759 – 5767. 54.

Jacobs, MD. and Harrison, SC .1998 . Structure of an IkappaBalpha/ NF-kappaB complex . *Cell* 95 (6), 749 – 58. 55.

Kagan, B. L., Baldwin, R. L., Munoz, D. and Wisnieski, B. J. 1992. Formation of Ion-Permeable Channels by Tumor Necrosis Factor-Alpha. *Science* 255, 1427-1430. 56.

Kang, O. H., Choi, J. G., Lee, J. H. and Kwon, D. Y. 2010. Luteolin Isolated from the Flowers of *Lonicera japonica* Suppresses Inflammatory Mediator Release by Blocking NF- κ B and MAPKs Activation Pathways in HMC-1 Cells. *Molecules* 15, 385-398. 57.

Karin, M. 2006. Nuclear factor- κ B in cancer development and progression. *Nature* 441, 431-436. 58.

Kelliher, MA., S. Grimm, Y. Ishida, F. Kuo, B., Z. Stanger. and P. Leder. 1998. The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunity.* 8,297 – 303. 59.

Kempuraj, D., Tagen, M., Iliopoulou, B. P., Clemons, A., Vasiadi, M., Boucher, W., House, M., Wolfberg, A. and Theoharides, T.C. 2008. Luteolin inhibits myelin basic protein-induced human mast cell activation and mast cell dependent stimulation of Jurkat T cells. *Br. J. Pharmacol.* 155, 1076-1084. 60.

Kiefer, F., Arnold, K., Kunzli, M., Bordoli, L. and Schwede, T. 2009. The SWISS -MODEL Repository and associated resources. *Nucleic Acids Res* .37,D387-392. 61.

Kim, J. S. and Jobin, C. 2005. The flavonoid luteolin prevents lipopolysaccharide-induced NF-kappaB signalling and gene expression by blocking IkappaB kinase activity in intestinal epithelial cells and bone-marrow derived dendritic cells. *Immunology.* 115, 375-387. 62.

Kim, K.Y., Kim, J.K., Jeon, J.H., Yoon, S.R., Choi, I. and Yang, Y. 2005. c-Jun N-terminal kinase is involved in the suppression of adiponectin expression by TNF- α in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* .327, 460 – 467. 63.

Kimata, M., Shichijo, M., Miura, T., Serizawa, I., Inagaki, N. and Nagai, H. 2005. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. *Clin. Exp. Allergy* .30,501-508. 64.

Kelliher, MA., S. Grimm, Y. Ishida, F. Kuo, B., Z. Stanger, and P. Leder. 1998. The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunology.* 8,297 – 303. 65.

Kempuraj, D., Madhappan, B.,

Christodoulou, S., Boucher, W., Cao, J., Papado-poulou, N., Cetrulo, C. L. and Theoharides, T. C. 2005. Flavonols inhibit pro-inflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. *Br. J. Pharmacol.* 145,934-944.

66. Kiviharju, T.M., Lecane, P.S., Sellers, R.G. and Peehl, D.M. 2002. Antiproliferative and proapoptotic activities of triptolide (PG490), a natural product entering clinical trials, on primary cultures of human prostatic epithelial cells. *Clin Cancer Res.* 8,2666 – 2674.

67. Kupchan, S. M., Court, W. A., Dailey, R.G. Jr., Gilmore, C.J., Bryan, R. F. 1972. Triptolide and triptolide novel antileukemic diterpenoid triepoxides from *Tripterygium wilfordii*. *J. Am. Chem. Soc.* 94, 7194 – 7195.

68. Lam, J., Nelson, C. A., Ross, F.P., Teitelbaum, S.L., Fremont, D.H. 2001. Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity. *J Clin Invest*, 108(7):971-9.

69. Laskowski, R. A., MacArthur, M.W., Moss, D. S., Smith, D. K. and Thornton, J. M. 1993. PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Cryst.* 26, 283-291.

70. Lee, K.Y., Chang, W., Qiu, D., Kao, P.N. and Rosen, G.D. 1999. PG490 (triptolide) cooperates with tumor necrosis factor- α to induce apoptosis in tumor cells. *J Biol Chem.* 274,13451 – 13455.

71. Lee, K.Y., Park, J.S., Jee, Y.K. and Rosen, G.D. 2002. Triptolide sensitizes lung cancer cells to TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by inhibition of NF- κ B activation. *Exp Mol Med.* 34,462-468.

72. Leuenroth, S.J. and Crews, C.M. 2005. Studies on calcium dependence reveal multiple modes of action for triptolide. *Chem Biol.* 12,1259 – 1268.

73. Li, Q., Verma, I.M. 2002. NF- κ B regulation in the immune system. *Nat Rev Immunol.* 2, 975.

74. Liacini, A., Sylvester, J. and Zafarullah, M. 2005. Triptolide suppresses proinflammatory cytokine-induced matrix metalloproteinase and aggrecanase-1 gene expression in chondrocytes. *Biochem Biophys Res Commun.* 327, 320 – 327.

75. Liu, Q., Chen, T. and Chen, H. 2004. Triptolide (PG-490) induces apoptosis of dendritic cells through sequential p38 MAP kinase phosphorylation and caspase 3 activation. *Biochem Biophys Res Commun.* 319,980-986.

76. Liu, K.Y., Wu, Y.C., Liu, I.M. and Cheng, J.T. 2008. Release of acetylcholine by syringin, an active principle of *Eleutherococcus senticosus*, to raise insulin secretion in Wistar rats. *Neurosci Lett.* 434(2),195-9.

77. Luftig, M., Prinarakis, E., Yasui, T., Tschritzis, T., Cahir-McFarland, E. 2003. Epstein-Barr virus latent membrane protein 1 activation of NF- κ B through IRAK1 and TRAF6. *Proc Natl Acad Sci U S A.* 100,15595 – 15600.

78. Madhusudan, Akamine, P., Xuong, N.H., Taylor, S. S. 2002. Crystal structure of a transition state mimic of the catalytic subunit of cAMP-dependent protein kinase. *Nat Struct Biol.* 9(4):273-7.

79. Manach, C. and Donovan, J. L. 2004. Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radic. Res.* 38,771-785.

80. Mattson, M. P. and Camandola, S. 2001. NF- κ B in neuronal plasticity and neurodegenerative disorders. *J. Clin. Invest.* 107, 247 – 254.

81. McWhirter, S.M., Pullen, S.S., Holton, J.M., Crute, J. J., Kehry, M.R., Alber, T. 1999. Crystallographic analysis of CD40 recognition and signaling by human TRAF2. *Proc Natl Acad Sci USA.* 96(15):8408-13.

82. Memon, R. A., Grunfeld, C., Moser, A. H. and Feingold, K. R. 1993. Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. *Endocrinology.* 132, 2246 – 2253.

83. Morris, A. L., MacArthur, M. W., Hutchinson, E. G. and Thornton, J. M. 1992. Stereochemical quality of protein structure coordinates. *Proteins.* 12, 345-364.

84. Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. 1998. Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. *J. Comput. Chem.* 19,1639-1662.

85. Muppidi, J.R., Tschopp, J. and Siegel, R.M. 2004. Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. *Immunity.* 21, 461 – 465.

86. Nikaido, H. 1993. Transport across the bacterial outer membrane. *J Bioenerg Biomembr.* 25, 581-589.

87. Pando, M.P., Verma, I.M. 2000. Signal dependent and independent degradation of free and NF- κ B-bound I κ B α . *J Biol Chem.* 275,21278 – 86.

88. Park, H. H., Lee, S., Son, H.Y., Park, S. B., Kim, M. S., Choi, E. J., Singh, T. S., Ha, J. H., Lee, M. G., Kim, J. E., Hyun, M. C., Kwon, T. K., Kim, Y. H. and Kim, S. H. 2008. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch. Pharm. Res.* 31,1303-1311.

89. Park, Y. C., Burkitt, V., Villa, A. R., Tong, L. and Wu, H. 1999. Structural basis for self-association and receptor recognition of human TRAF2. *Nature.* 398(6727):533-8.

90. Peitsch, M. C. 1995. Protein modeling by E-mail. *Nat. Biotechnol.* 13, 658-660.

91. Peraldi, P., Hotamisligil, G. S., Buurman, W. A., White, M. F. and Spiegelman, B. M. 1996. Tumor necrosis factor (TNF)- α inhibits insulin signaling through stimulation of the p55TNF receptor and activation of sphingomyelinase. *J. Biol. Chem.* 271, 13018 – 13022.

92. Pruzanski, W., Vadas, P., Browning, J. 1993. Secretory non-pancreatic group II phospholipase A2: role in physiologic and inflammatory processes. *J. Lipid Mediat.* 8, 161 – 167.

93. Riaz, A. M., Walter, M. H., Arthur, H. M., Taisuke, S., Yoshikazu, U., John, F., Judy, K. S., Carl, G. and Kenneth, R. F. 1998. Endotoxin and cytokines increase hepatic sphingolipid biosynthesis and produce lipoproteins enriched in ceramides and sphingomyelin. *Arterioscler. Thromb. Vasc. Biol.* 18, 1257 – 1265.

94. Rink, L. and H. Kirchner. 1996. Recent Progress in the Tumor Necrosis Factor -Alpha Field. *Int. Arch. Allergy Immunol.* 111,199.

95. Ross, R. 1999. Atherosclerosis – an inflammatory disease. *N. Engl. J. Med.* 340, 115.

96. Saghizadeh, M., Ong, J.M., Garvey, W.T., Henry, R. R. and Kern, P.A. 1996. The expression of TNF α by human muscle. Relationship to insulin resistance. *J Clin Invest.* 15;97(4):1111-6.

97. Schomer-Miller, B., Higashimoto, T., Lee, Y. K. and Zandi, E. 2006. Regulation of I κ B kinase (IKK) complex by IKK γ -dependent phosphorylation of the T-loop and C terminus of IKK β . *J Biol Chem.* 281, 15268-15276.

98. Schwede, T., Kopp, J., Guex, N. and Peitsch, M. C. 2003. SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Re.* 31,3381 – 3385.

99. Shamon, L.A., Pezzuto, J.M., Graves, J.M., Mehta, R.R., Wangcharoentrakul, S., Sangsuwan, R., Chaichana, S., Tuchinda, P., Cleason, P. and Reutrakul, V. 1997. Evaluation of the mutagenic, cytotoxic, and antitumor potential of triptolide, a highly oxygenated diterpene isolated from *Tripterygium wilfordii*. *Cancer Lett.* 112,113 – 117.

100. Shi, C.S. and Kehrl, J.H. 2003. Tumor necrosis factor (TNF)-induced germinal center kinase related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13 – Uev1A/TNF receptor-associated factor 2 (TRAF2). *J Biol Chem.* 278, 15429 – 15434.

101. Shimada, K., Miyazaki, T. and Daida, H. 2004. Adiponectin and atherosclerotic disease. *Clin Chim Acta.* 344,1 – 12.

102. Sica, A., Wang, J. M., Colotta, F., Dejana, E., Mantovani, A., Oppenheim, J. J., Larsen, C. G., Zachariae, C. O. and Matsushima,

K. 1999. Monocyte chemotactic and activating factor gene expression induced in endothelial cells by IL-1 and tumor necrosis factor. *The Journal of Immunology*. 144, 3034 – 3038.

103. Skoog, T., Dichl, W., Boquist, S., Skoglund-Andersson, C., Karpe, F., Tang, R., Bond, MG., de Faire, U., Nilsson, J., Eriksson, P. and Hamsten, A. 2002. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J*. 23,376-383.

104. Stefan, N., Bunt, J., Slabe, A., Funahashi, T., Matsuzawa, Y. and Tataranni, P. 2002. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *J. Clin Endocrinol Metab*. 87,4652 – 4656.

105. Sun, SC., Ganchi, PA., Ballard, DW., Greene, WC. 1993. NF-kappa B controls expression of inhibitor I kappa B alpha: evidence for an inducible autoregulatory pathway. *Science*. 259,1912 – 5.

106. Tak, P. P. and Firestein, G. S. 2001. NF- B: a key role in inflammatory diseases. *J. Clin. Invest*. 107,7 – 11.

107. Tang, G., Minemoto, Y., Dibling, B., Purcell, NH., Li, Z., Karin, M. and Lin, A. 2001. Inhibition of JNK activation through NF-kappaB target genes. *Nature*. 414, 313 – 317.

108. Tao, X., Davis, L. S. and Lipsky, P. E. 1991. Effect of an extract of the Chinese herbal remedy *Tripterygium wilfordii* Hook F on human immune responsiveness. *Arthritis Rheum*. 34, 1274 – 1281.

109. Tao, X., Cush, JJ., Garret, M. and Lipsky, PE. 2001. A phase I study of ethyl acetate extract of the chinese antirheumatic herb *Tripterygium wilfordii* hook F in rheumatoid arthritis. *J Rheumatol*. 28,2160 – 7.

110. Tao, X., Younger, J., Fan, F. Z., Wang, B. and Lipsky, P. E. 2002. Benefit of an extract of *Tripterygium wilfordii* Hook F in patients with rheumatoid arthritis. *Arthritis Rheum*. 46,1735 – 1743.

111. Theoharides, T. C., Dimitriadou, V., Letourneau, R. J., Rozniecki, J. J., Vliagoftis, H. and Boucher, W. S. 1993. Synergistic action of estradiol and myelin basic protein on mast cell secretion and brain demyelination: changes resembling early stages of demyelination. *Neuroscience*. 57, 861 – 871.

112. Tracey, K. J. and Cerami, A. 1990. Metabolic responses to cachectin/TNF. A brief review. *New York Academy of Sciences*. 587,325-331.

113. Tracey, KJ. and Cerami, A. 1994. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. *Annu Rev Med*. 45,491-503.

114. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R. and Verma, I. M. 1996. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science*. 274, 787-789.

115. Verma, IM., Stevenson, JK., Schwarz, EM., Van Antwerp, D., Miyamoto, S. 1995. "Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation". *Genes Dev*. 9 (22), 2723 – 35.

116. Vivarelli, M. S., McDonald, M. Miller, N. Cusson, M. Kelliher. and R. S. Geha. 2004. RIP links TLR4 to Akt and is essential for cell survival in response to LPS stimulation. *J. Exp. Med*. 200:399 – 404.

117. Wallace, A. C., Laskowski, R. A. and Thornton, J. M. 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng*. 8, 127-134.

118. Wei, YS. and Adachi, I. 1991. Inhibitory effect of triptolide on colony formation of breast and stomach cancer cell lines. *Chung Kuo Yao Li Hsueh Pao*. 12:406 – 410.

119. Wu, CJ., Conze, DB., Li, T., Srinivasula, SM., Ashwell, JD. 2006. Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation. *Nat Cell Biol*. 8:398-406.

120. Xagorari, A., Papapetropoulos, A., Mauromatis, A., Economou, M., Fotsis, T. and Roussos, C. 2001. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J. Pharmacol. Exp. Ther*. 296, 181 – 187.

121. Yang, J., Cron, P., Good, VM., Thompson, V., Hemmings, BA. and Barford, D. 2002. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat Struct Biol*. 9(12):940-4.

122. Ye, H., Park, YC., Kreishman, M., Kieff, E. and Wu, H. 1999. The structural basis for the recognition of diverse receptor sequences by TRAF2. *Mol Cell*. 4(3):321-30.

123. Zhang, L. X., Pan, D. J., Zhang, W. J., Wu, H. Y. and Lou, W. P., 1986. Inhibitory effect of tripterygium on lymphocyte proliferation in mice. *Acta Pharm. Sin* 7, 85 – 88.