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Design and Synthesis of Heterocyclic Curcumin Analogues as Filarial Topoisomerase II Inhibitors

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ABSTRACT: A series of novel heterocyclic curcumin analogues (substituted cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one derivatives 3-24), were designed and synthesized via condensation of different benzaldehydes and dehydroacetic acid. A new way for modification of curcumin has been described in order achieves enhanced pharmacological properties. Synthesized derivatives were also evaluated for their inhibitory activity against filarial topoisomerase II enzyme. All the compounds screened against Topoisomerase II exhibited excellent inhibition upto percentage inhibition more than 95%. Further the structure–activity relationships of the cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one derivatives (3-24) reveals that among the synthesized compounds, the nitro substituted chalcones derivatives 5 and 8 were the most active compounds by showing significant inhibition of *S. cervi* Topoisomerase II activity upto more than 95%.

Keywords: Curcumin; Anticancer; Topoisomerase inhibition; Chalcone and heterocycle.

INTRODUCTION: Curcumin is the active constituent of Indian traditional medicine turmeric and is very well known for its diverse range of pharmacological properties particularly anti-inflammatory and anticancer. Due to its pharmacological importance Curcumin is very fascinating molecule for organic and medicinal chemist. Walking on similar track we have proposed here a new way of modifying the curcumin nucleus to get anticancer property. Cancer is one of the major challenges for medicinal chemists and cause for millions of deaths worldwide. In spite of the recent progress and the development cancer chemotherapy, there is still need for new compounds of therapeutic interest to bring this disease under control. In the world of caner chemotherapeutics natural products are very valuable molecules. A large number of modern drugs have been developed from natural sources, especially from plants.[1] A variety of naturally occuring compounds such as curcumin, paclitaxol, vinblastin, combretastatin A-4, desmosdumotin C and colchicine are well known anticancer agents. Natural product derived or an inspired molecule forms a large group of compounds with anticancer activity. Among them curcumin is the compound possessing a large number of biological activities and is most abundant in nature. It is the yellow pigment extracted from the rhizoma of *Curcuma longa*, is the pharmacologically active substance of turmeric. By tradition, turmeric has been used for many ailments, particularly as an anti-inflammatory agent, and curcumin has been identified as the active principle of turmeric.[2] Curcumin is non-toxic and has a variety of positive pharmacological effects as anti-inflammatory, anti-oxidative and anti-septic properties have been reported and displayed good pharmacological effect in cardiovascular diseases, sporadic Alzheimer's disease (AD), sarcopenia, type II diabetes, arthrosis and arthritis among others.[3-5]

Regardless of its important place in field of drug discovery, curcumin has not yet been approved as a therapeutic agent, and the relative bioavailability and solubility of curcumin has been highlighted as a major problem behind this issue.[6] Eventually this drawback render this molecule a possible anti-cancer drug, as both chemo preventive and chemotherapeutic. This evidence has strongly suggests that curcumin can be considered a promising tool for cancer therapy and need some modification in order to overcome theses drawbacks.

Among the various natural anticancer molecules, chalcones contains potentially important group of structurally effective feature bearing ketone functionality and an unsaturated group.[7-8] These are in conjugated arrangement among the various naturally occurring as well as synthetic anticancer agents. These class of compounds are well known for their tumorreudcing and antiproliferative activities.[9-10] Any change in the three carbon propenone skeleton is known to lose the biological activities. Incorporating heterocyclic rings in chalcone core structure has been employed by medicinal chemists to enhance the pharmacological properties,[11-12] *viz.* some coumarin-based compounds psorospermin (b), have been reported to display a good cytotoxic activity, exerting a good anticancer and topoisomerase II inhibitor activity. [13]

DNA topoisomerase II is a enzyme that controls DNA topology by transient cleavage of the DNA double helix. The non covalent interaction of protein with DNA is the key step in the topoisomerase II catalytic cycle. Under physiological conditions, DNA replication, repair, and transcription processes are significantly controlled by Topoisomerase II. Among the various enzymes identified as target against parasitic diseases, DNA topoisomerases have attracted medicinal chemists as a novel target for antifilarial drug development.[14] DNA topoisomerases are the enzymes required for the replication, transcription and recombination of DNA.[15] These enzymes play crucial roles in the organization of DNA within the cell nucleus as well as in its structure and function.

Now a days in the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores may lead to compounds with interesting biological profiles.[16-18] Original curcumin molecule mainly has two pharmacophoric regions as indicated by A and B (Figure 1). Earlier studies in modification of curcumin revealed broadly 4 types of modification separately in part A and B (Figure 1). A careful survey of literature reveals that the curcumin nucleus has been modified in many ways. One way to modify curcumin structure was achieved by replacing 1,3-dicarbonyl with cyclic ring (path A).[19-20] Some modifications were reported via varying the aromatic ring with heterocyles (path B).[21] In vitro studies showed that these compounds had better inhibitory properties against Aβ aggregation than curcumin.[22-23]

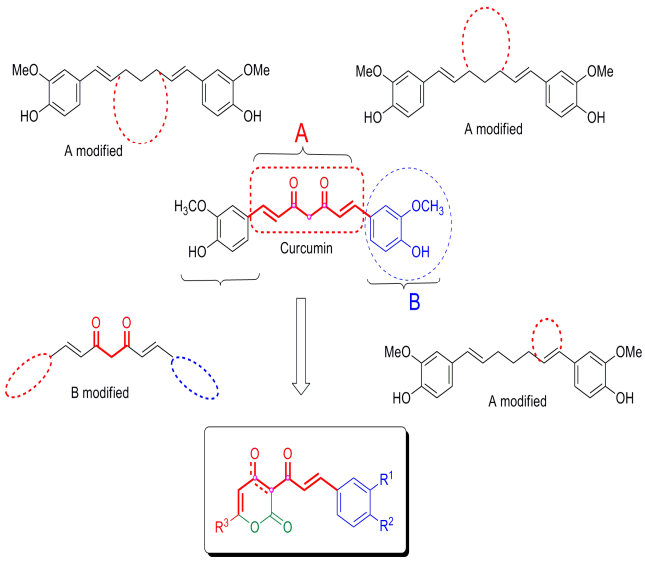


Figure 1: Various sites of modification in curcumin nucleus.

This combination rationale has been used in our laboratory to synthesized 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one analogues by incorporating conjugated 1,3 dicarbonyl group from curcumin and pyran-2-one moiety from different biologically active natural product nucleus as novel class of cytotoxic agents. (Figure 2) We thought to synthesize the chalcone analogue consisting of both the phrmacophore and to see their cytotoxic activity in various in vitro cancer cell lines and filarial topoisomerase-II activity.

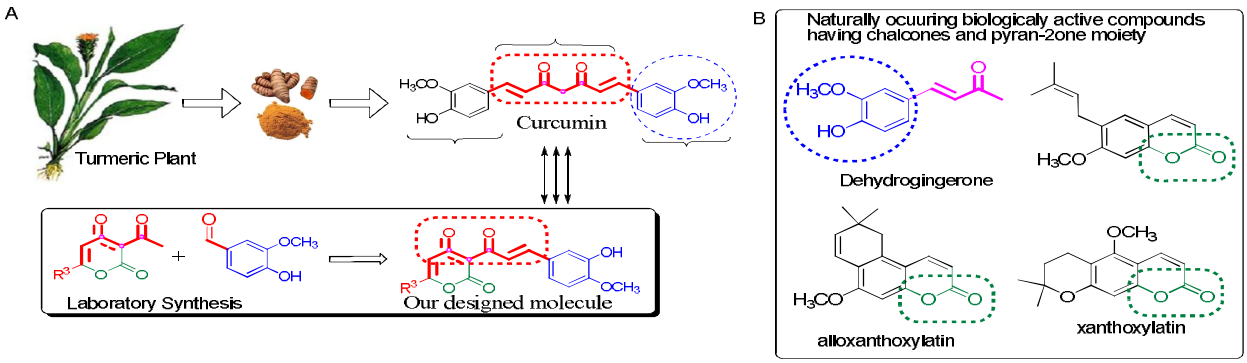


Figure 2: (a) Designing of curcumin type chalcone analogues. (b) Some naturally occurring biologically important chalcones.

MATERIAL AND METHODS: Unless otherwise specified all the reagents and catalysts were purchased from Sigma-Aldrich and were used without further any purification. The common solvents were purchased from Ranbaxy. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. Chromatographic purification of products was accomplished using flash chromatography on 230-400 mesh silica gel. Reactions were monitored by thin-layer chromatography (TLC) on 0.25mm silica gel plates visualized under UV light, iodine or $KMnO_4$ staining. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-200 MHz Spectrometer. Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hz. IR spectra were recorded on a FTIR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Mass spectra (ESIMS) were obtained by micromass quattro II instrument.

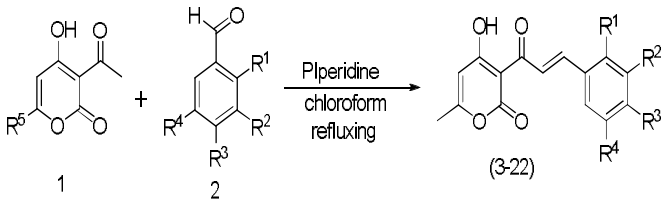
General procedure for synthesis of chalcone analogues (3-22): Chalcone analogues (3-22) were synthesized via aldol condensation of substituted benzaldehyde and dehydro acetic acid. In dry chloroform substituted benzaldehyde (1.0 mmol), dehydroacetic acid (1.0 mmol) in the presence of catalytic pyrrolidine (20 mol %) was taken. Reaction was stirred at room temperature for 2 hrs leading to generation of chalcone. Progress of reaction was monitored by TLC. After completion of reaction solvent was evaporated under the reduced pressure and residue was extracted with ethyl acetate and water. The organic layer was separated and dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under vacuum on a rotary evaporator.

Estimation of DNA Topoisomerase II inhibitory activity of compounds: The reaction catalyzed by DNA topoisomerase II was estimated as reported earlier (Pandya et al. 1999).**[1]** The reaction mixture in a final volume of 20 µl contained 50 mM Tris. HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 1 mM ATP, 0.1 mM EDTA, 0.5 mM dithiothreitol (DTT), 30 µg/ml bovine serum albumin (BSA), 0.25 µg pBR322 DNA and enzyme protein. The reaction was carried out at 37º C for 30 min. and stopped by adding 5 µl stop buffer. The samples were subjected to electrophoresis on 1% agarose gel in Tris-acetate buffer for 18 h at 20 V. Gels were stained with ethidium bromide (0.5 µg/ml) and visualized and photographed on a GDS 7500 UVP Trans illuminator (Ultraviolet Products, UK). The effect of inhibitors on the enzyme activity was measured by incubating enzyme with the inhibitor for 10 min. at 37 º C and starting the reaction by addition of pBR322 DNA. The percentage inhibition was measured by micro densitometry of the gel with the gel base/gel blot Progel analysis software program

Spectroscopic and analytical details of 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one (3): White solid; mp 146^{0}C; ν_{max}(KBr) 3424, 3083, 1725, 1626, 1326, 1236 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.36 (1H, d, *J*= 15.75Hz); 8.01 (1H, d, *J*= 15Hz); 7.27-7.69 (2H, m), 7.45-7.42 (3H, m), 5.98 (1H, s); 2.30 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 191.0, 182.4, 166.5, 162.1, 150.5, 148.6, 131.6, 129.8, 127.5, 123.21 120.6, 116.1, 111.4, 103.1, 98.9, 20.7.; MS (ES): m/z (%) = 257 (100) $[M+1]^+$; Ana. calcd. for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; Found: C, 70.28; H, 4.69%

RESULTS AND DISCUSSION:

Chemistry: In present work, we describe the synthesis as well as Filarial Topoisomerase II inhibitory activity of 22 substituted natural product inspired chalcone type analogues (Table 1 & 2). Synthesized analogues bearing different functional groups at phenyl ring in an attempt to optimize the anticancer activity and potentially gain insight into the structure activity relationship of 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one and its derivatives. The reaction scheme for the synthesis of designed analogue is shown schematically in Figure 2. Compounds (3-22) were synthesized from dehydroacetic acid (1) and substituted benzaldehydes (2) via condensation reaction using pyridine as base. Both the reactants were mixed in anhydrous chloroform and reaction was allowed to reflux for appropriate time period upto completion of reaction. After completion of reaction desired products were isolated by only filtration. Negligible or no chromatography required for purification. All the compounds synthesized were confirmed by spectroscopic (^1H NMR and ^{13}C NMR) and spectrometric (mass) data.[17]



Scheme 1: synthesis of various chalcones derivatives.

Biological activity:

Topoisomerase-II inhibitory activity: The ten compounds (4, 5, 6, 7, 8, 14, 16, 17, 18 and 19**)** selected on the basis of maximum inhibitory effect against cancer cell lines were biologically screened against *S.*

cervi Topoisomerase II enzyme. Results indicated that compounds screened were found to be active against Topoisomerase II enzyme (Figure 3) with percentage inhibition in range of 30-95%. Inhibition of Topoisomerase II activity was examined by studying the enzyme-mediated supercoiled pBR322 relaxation.

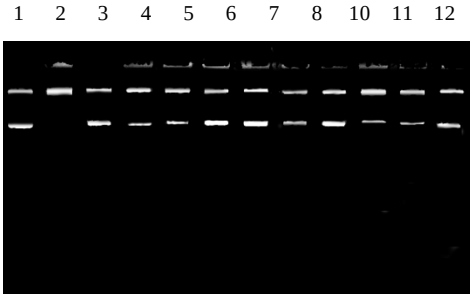


Figure 3: Gel mobility shift assay of *S. cervi* topoisomerase II. Lane 1: pBR322 (0.25µg) alone; lane 2: pBR322 + *S. cervi* Topo II; Lane 3: pBR322 + *S. cervi* Topo II + Comp. 7 (40 µg); Lane 4: pBR322 + *S. cervi* Topo II + Comp. 4 (40 µg); Lane 5: pBR322 + *S. cervi* Topo II + Comp. 6 (40 µg); Lane 6: pBR322 + *S. cervi* Topo II + Comp. 8 (40 µg); Lane 7: pBR322 + *S. cervi* Topo II + Comp. 5 (40 µg); Lane 8: pBR322 + *S. cervi* Topo II + Comp. 9 (40 µg); Lane 9: pBR322 + *S. cervi* Topo II + Comp. 18 (40 µg); Lane 10: pBR322 + *S. cervi* Topo II + Comp. 14 (40 µg); Lane 11: pBR322 + *S. cervi* Topo II + Comp. 19 (40 µg); Lane 12: pBR322 + *S. cervi* Topo II + Comp. 23 (40 µg).

Table 1: Topoisomerase II inhibitory activity of test compounds against filarial parasite *Setaria cervi*.

Lane no.	Lane contents	% inhibition
1	pBR 322 DNA only (Control)	-
2	pBR 322 DNA only + *S. Cervi* DNA pol. II (Experimental)	-
3	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 7 (40 µg)	95
4	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 4 (40 µg)	50
5	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 6 (40 µg)	50
6	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 8 (40 µg)	95
7	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 5 (40 µg)	95
8	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 9 (40 µg)	75
9	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 18 (40 µg)	95
10	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 14 (40 µg)	30
11	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 19 (40 µg)	30
12	pBR 322 DNA only + *S. Cervi* DNA pol. II + Comp. 23 (40 µg)	90

Table 2: Synthesis and in vitro anticancer activity of synthesized 3-cinnamoyl-4-hydroxy-6-methyl-2H-pyran-2-one derivatives.

Compound	R^1	R^2	R^3	R^4	Yield
3	H	H	H	H	88
4	H	H	OCH_3	H	91
5	H	OCH_3	H	H	90
6	H	OCH_3	OCH_3	H	90
7	H	H	NO_2	H	89
8	NO_2	H	H	H	83
9	H	H	N,N-$(CH_3)_2$	H	92
10	H	Cl	H	H	91
11	H	H	Cl	H	92
12	H	Cl	H	3-Cl	90
13	H	OH	OCH_3	H	86
14	H	H	OCH_2Ph	H	87
15	H	OH	H	H	84
16	H	H	OH	H	82
17	H	H	F	H	94
18	OCH_3	H	H	H	90
19	3-Cl	H	H	H	92
20	H	Br	H	H	90
21	OCH_3	H	H	OCH_3	88
22	OCH_3	OCH_3	H	OCH_3	86
23	H	NO_2	H	H	91
24	H	OCH_3	OCH_2Ph	H	90

Structure activity relationship of all the 10 screened compounds have given clear indication that nitro substitution at phenyl ring and methoxy substitutions are very crucial in exerting topoisomerase II inhibitory activity against filarial parasite *Setaria cervi*. Most of the active compounds (8, 11, 12, 13 and 14) either having nitro group or methoxy substitution at phenyl ring. Identification of these novel and chemically diverse Topoisomerase II enzyme inhibitors provides initial leads for optimization into more potent and efficacious drug candidates to treat filarial infection.

CONCLUSION: In conclusion, we have designed and synthesized 22 heterocyclic curcumin analogues and they were screened for topoisomerase inhibitory activity. All the analogues were synthesized via condensation reaction using piperidine as organic base. Ten coumpounds which were tested against filarial topoisomerase-II are found to be posses promising activity with percentage inhibition of more than 95%. We further established SAR study to rationalize the biological activity. Our findings suggest that the presence of nitro and methoxy group in phenyl ring of chalcone moiety is prime factor for the pharmacological activity of compounds. Our study is very effective preliminary study in anticancer drug discovery project and these results can help to search for a potential drug candidate in future.

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REFERENCES:

1. Kyoko, N. G., Peng, T. H., C. Y., Bastow, K. F., Wu, J. H. and Lee, K. H. (2007) Antitumor Agents 259. Design, Syntheses, and Structure−Activity Relationship Study of Desmosdumotin C Analogs, *J. Med. Chem.*, 50, 3354-3358.
2. Kocaadom B., Sanlier N., Curcumin, an active component of turmeric (Curcuma longa), and its effects on health (2015) Critical Reviews in food science and nutrition, 2889-2895.
3. Devassy, J., Nwachukwu, I. and Jones, P. (2015). Curcumin and cancer: Barriers to obtaining a health claim. Nutr. Rev. 73, 3,155-165
4. Shishodia S., Chaturvedi M. M., Aggarwal B. B. (2007) Role of Curcumin in cancer therapy, *Curr. Probl. Cancer*, 31, 243.
5. Deogade, S. and Ghate, S. (2015). Curcumın: Therapeutıc applıcatıons in systemıc and oral health. Int. J. Biol. Pharm. Res. 6 (4), 281–290.
6. (a) Sharma, R. A., Gescher; A. J. and Steward, W. P. (2005) Curcumin-The story so Far, *European Journal of Cancer*, 41, 1955 . (b) Chattopadhyay, I., Biswas, K., Bandyopadhyay, U. and Banerjee R. K. (2004) Turmeric and curcumin: Biological actions and medicinal applications, *Current Science*, 87, 44.
7. Sharma, A., Chakravarti, B., Gupt, M. P., Siddiqui, J, A., Konwar, R. and Tripathi, R. P. (2010) Synthesis and anti breast cancer activity of biphenyl based chalcones Bioorg. Med. Chem., 13, 4711-4720.
8. Aponte, J. C., Vrastegui, M., Malaga, M., Zimic, M., Quiliano, M., Vaisberg, A. J., Gilman R. H. and Hammond G. B. (2008) Synthesis, Cytotoxicity, and Anti-*Trypanosoma cruzi* Activity of New Chalcones, *J. Med. Chem.*, 51, 6230.
9. Nowakowska, Z. (2007) A review of anti-infective and anti-inflammatory chalcones, *Eur. J. Med. Chem.*, 42, 125.
10. Cabrera, M., Simoens, M., Falchi, G., Lavaggi, M. L., Piro, O. E., Castellano, E. E., Vidal, A., Azqueta, A., Monge, A., Lopez de Cerain, A., Sagrera, G., Seoane, G., Cerecetto, H. and Gonzalez, M. (2007) Synthetic chalcones, flavanones, and flavones as antitumoral agents: Biological evaluation and structure–activity relationships, *Bioorg. Med. Chem.*, 15, 3356.
11. Ni, L., Meng, C. Q. and Sikorski, J. A. (2004) *Expert Opin. Ther. Pat.*, 14, 1669.
12. Meng, C. Q., Zheng, X. S, Ni, L., Ye, Z., Simpson, J. E., Worsencroft, K. J., Hotema, M. R., Weingarten, M. D., Skudlarek, J. W., Gilmore, J. M., Hoong, L. K., Hill, R. R., Marino, E. M., Suen, K. L., Kunsch, C., Wasserman, M. A. and Sikorski, J. A. (2004) Discovery of novel heteroaryl-substituted chalcones as inhibitors of TNF-α-induced VCAM-1 expression, *Bioorg. Med. Chem. Lett.*, 14, 1513.
13. Adams, B. K., Ferstl, E. M., Davis, M. C., Herold, M., Kurtkaya, S., Camalier, R. F., Hollingshead, M. G., Kaur, G., Sausville, E. A., Rickles, F. R., Snyder, J. P., Liottab D. C. and Shojia, M. (2004) Synthesis and biological evaluation of novel curcumin analogs as anti-cancer and anti-angiogenesis agents, *Bioorg. Med. Chem.*, 12, 3871.
14. Katiyar, S. B., Bansal, I., Saxena, J. K. and Chauhan, P. M. S. (2005) Syntheses of 2,4,6-trisubstituted pyrimidine derivatives as a new class of antifilarial topoisomerase II inhibitors, *Bioorg. Med. Chem. Lett.*, 15, 47.
15. Wei, P., Zhang, X., Tu, S., Yan S. Ying, H. and Ouyang, P. (2009) New potential inhibitors of DNA topoisomerase. Part II: Design and synthesis of α-lapachone derivatives under microwave irradiation, *Bioorg. Med. Chem. Lett.*, 19, 828.
16. Justine, L.; Delgado, Hsieh C.-M., Chan N. L., Hiasa H.; (2018); Topoisomerases as anticancer targets; 475 (2), 373-398.
17. R. Kumar, L. Coronel, C. F. Cheok; (2018) Mitochondrial uncoupling reveals a novel therapeutic opportunity for p53-defective cancers; 9, 3931.
18. Hu W., Huang X.-S., Wu J.-F., Yang L., Zheng Y.-T., Shen Y.-M., Li Z.-Y., and Li X. (2018) Discovery of Novel Topoisomerase II Inhibitors by Medicinal Chemistry Approaches; J. Med. Chem. DOI: 10.1021/acs.jmedchem.7b01202.
19. Franco-Ulloa S., Sala G. L., Miscione G. P., Vivo M. D. (2018) Novel Bacterial Topoisomerase Inhibitors Exploit Asp83 and the Intrinsic Flexibility of the DNA Gyrase Binding Site; Int. J. Mol. Sci.; 19, 453.
20. Kumar, A., Sharma, S., Tripathi, V. D., Maurya, R. A., Srivastava, S. P., Bhatia, G., Tamrakar, A. K. and Srivastava, A. K. (2010) Design and synthesis of 2,4-disubstituted polyhydroquinolines as prospective antihyperglycemic and lipid modulating agents, *Bioorg. Med. Chem.*, 18, 4138.
21. Zhao, C., Yang, J., Wanga, Y., Liang, D., Yang, X., Li, X., Wua, J., Wua, X., Yang, S., Li, X. and Liang, G. (2010) Synthesis of mono-carbonyl analogues of curcumin and their effects on inhibition

of cytokine release in LPS-stimulated RAW 264.7 macrophages, *Bioorg. Med. Chem.*, 18, 2388-2393.

22. Zhang, Q., Zhong, Lin-Na Yan Y., Sun, X., Gong, T. and Zhang, Z. R. (2011) Synthesis and preliminary evaluation of curcumin analogues as cytotoxic agents, *Bioorg. Med. Chem. Lett.*, 21, 1010-1014.

23. Chen, S. Y., Chen, Y., Li, Y. P., Chen, S. H., Tan, J. H., Ou, T. M., Gu, L. Q. and Huang, Z. S. (2011) Design, synthesis, and biological evaluation of curcumin analogues as multifunctional agents for the treatment of Alzheimer's disease, *Bioorg. Med. Chem.*, 19, 5596-5604.