# Understanding molecular mechanisms of prostate cancer biology using tetracyclic triterpenoid derivatives from *Boswellia serrata* as cell signaling modulators.

Research Proposal
submitted to
***Department of Biotechnology***
Government of India
New Delhi

Dr. Latha Diwakar
Vittal Mallya Scientific Research Foundation
93/3 & 94/5, 23rd cross, 29th main
BTM II Stage, Bangalore-560076
www.vmsrf.org
September 2014

**PROFORMA – I**

**PROFORMA FOR SUBMISSION OF PROJECT PROPOSALS ON RESEARCH AND DEVELOPMENT, PROGRAMME SUPPORT**

*(To be filled by the applicant)*

**PART I: GENERAL INFORMATION**

1. Name of the Institute/University/Organisation submitting the Project Proposal:

**Vittal Mallya Scientific Research Foundation**.

2. State: **Karnataka** 3. Status of the Institute: **Nonprofit R& D**
*(Please see Annexure-I)*

4. Name and designation of the Executive Authority of the Institute/University forwarding the application:

**Dr. Anil Kush, Research Director, Vittal Mallya Scientific Research Foundation.**

5. Project Title: **Understanding molecular mechanisms of prostate cancer biology using tetracyclic triterpenoid derivatives from *Boswellia serrata* as cell signaling modulators.**

6. Category of the Project (Please tick): R&D

7. Specific Area *(Please see Annexure - II):* **Basic science**

8. Duration: **Three Years**

9. Total Cost (Rs.) **77, 54, 800 /- Rs.**

10. Is the project Single Institutional or Multiple-Institutional (S/M): **Single**

11. If the project is multi-institutional, please furnish the following:

Name of Project Coordinator: Not applicable Affiliation: Not applicable

Address: Not applicable

12. Scope of application indicating anticipated product and processes

Cell signaling plays an important in role in the disease progression of chronic inflammatory disorder. The role of inflammatory cytokines such as interleukin and chemokines, inflammatory enzymes such as cyclooxygenase (COX-1 & 2) and 5-lipoxygenase (5-LOX) are well documented in the pathogenesis of prostate cancer biology. Therefore inhibitors of lipoxygenases which modulate Akt pathways have been reported potential molecules for prostate cancer in

many studies as expression of Akt is high in these cells. In present project we want to use 3-oxo-tirucallic acid since these are known to be Lipoxygenase inhibitors and aim at understanding molecular mechanisms of apoptosis in prostate cancer cells.

13. Project Summary (Not to exceed one page. Please use separate sheet).

Prostate cancer is the most frequently diagnosed malignancy in men. It is hormone sensitive cancer influenced by androgens mainly from testis, adrenal glands and other tissues like prostrate. Most relevant issue in prostate cancer drug discovery is ability to target both androgens sensitive and insensitive cells to avoid reoccurrence which can be achieved by finding compounds acting on multiple targets. Emerging studies have revealed that prostate cancer cells continuously generate metabolites from arachidonic metabolism. The pathway transforms arachidonic acid in to hydroperoxy-eicosatetraenoic acids (HETE) is mediated by lipoxygenases. The most prominent enzyme is 5-lipoxygenase leading to formation of leukotrienes. The formation of prostagladins from arachidonic acid is by a group of enzymes called cyclooxygenase, which are further sub divided into cyclooxygenase-1 which is constitutive and cyclooxygenase-2 is inducible.

*Boswellia serrata*, known in the vernacular as "Salai guggal", gum has been used in the Ayurvedic system of medicine for the management of rheumatism, respiratory diseases, and liver disorders. The gum resin of *Boswellia serrata* typically contains boswellic acids and other triterpenoids like tirucallic acids of which 3-oxo-tirucallic acid (3-oxo-TA) is the major constituent. The Akt signalling pathway plays a key role in the regulation of cell division and survival of cancer cells. Loss of phosphatase and tensin homolog deleted on chromosome ten (PTEN), a negative regulator of Akt activation, results in sustained expression of Akt in prostate cancer cells. Therefore inhibitors of Akt pathway can be potential therapeutic targets for prostate cancer. Studies on Tirucallic acid have shown that they are potential 5-LOX inhibitors and inhibited Akt signalling pathways inducing apoptosis in prostate cancer tumours.

In present study we propose to use these modified derivatives of tirucallic acid which shows better cytotoxic activity than 3-oxo-TA in regulating Akt pathway. Apart from Akt pathway we also want to study the NFkB pathway as it is key signaling pathway for 5-LOX and Cox-2 expression (Fig.1). Finally we want to establish apoptotic ability of these derivatives in PC-3 and LNCaP cell line which are androgen sensitive and insensitive prostate cancer cells. The proposed study would open new opportunities to make novel anticancer molecules based on the better understood mechanism of action hence would be more efficient, potent in both androgen sensitive and insensitive. The study is focused on scientific information and validation to traditional knowledge using natural compounds and moves a step further to develop new therapeutic molecules for future.

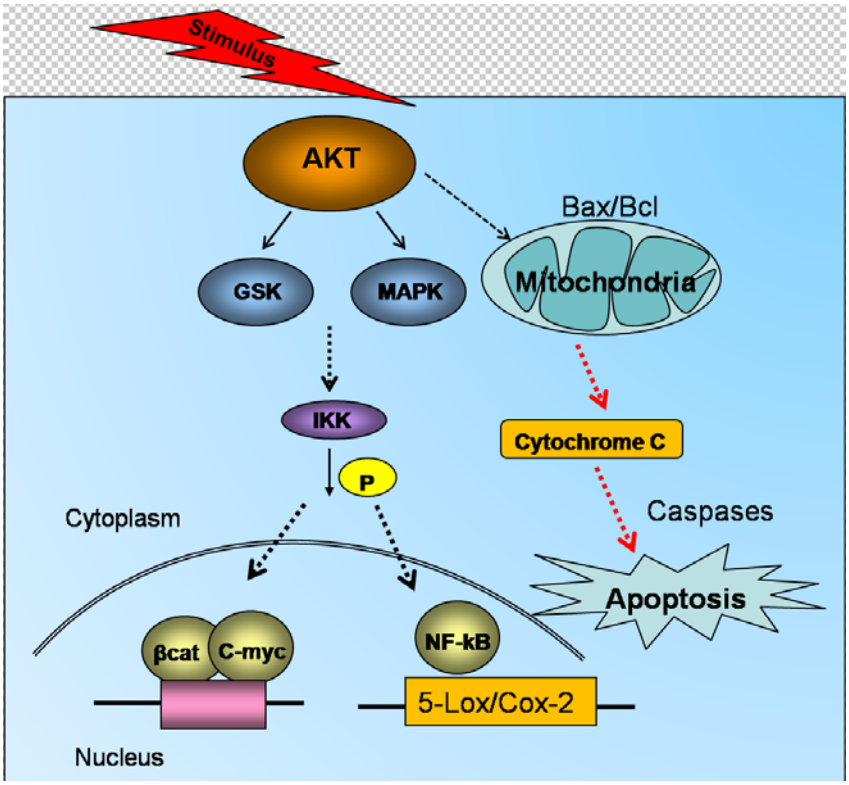


**Figure.1 Proposed molecular mechanism in cell signaling to study the effect of 3-oxo-TA derivatives in Prostate cancer**.

## PART II: PARTICULARS OF INVESTIGATORS

(*One or more co-investigators are preferred in every project. Inclusion of co-investigator(s) is mandatory for investigators retiring before completion of the project*)

**Principal Investigator:**

14. Name: Latha Diwakar

Date of Birth: 30th November 1973 Sex (M/F): Female

Designation: Scientist

Department: Biology

Institute/University: Vittal Mallya Scientific Research Foundation

Address: 94/3 & 94/5, 23rd cross, 29th Main, BTM II Stage, Bangalore, Karnataka

PIN: 560 076 Telephone: 080-26687216, 26687223, 26687183 Fax: 080-26687170

Email: latha@vmsrf.org.

Number of research projects being handled at present: one

**Co-Investigator**

15. Name: Dr. Venkatesham Uppala Date of Birth: 15/06/1972 Sex (M/F):Male

Designation: Research Scientist Department: Chemistry

Institute/University: Vittal Mallya Scientific Research Foundation
Address : #94/3 & 94/5, 23rd Cross, 29th Main, BTM II Stage, Bangalore, Karnataka

PIN: 560 076 Telephones: 080-26687216, 26687223, 26687183 Fax: 080-26687170

E-mail: venkatesham@vmsrf.org

Number of Research projects being handled at present: 0

**Co-Investigator**

16. Name: Dr.Anil Kush. Date of Birth: 13-9-1956 Sex(M/F) : Male

Designation: Research Director Department: Biology

Institute/University: Vittal Mallya Scientific Research Foundation

Address: #94/3 & 94/5, 23rd Cross, 29th Main, BTM II Stage, Bangalore, Karnataka
PIN: 560 076 Telephone: 080-26687216, 26687223, 26687183 Fax: 080-26687170

E-mail: anil.kush@gmail.com

Number of Research projects being handled at present: one

***Note:*** *Use separate page, if more investigators are involved*

## PART III: TECHNICAL DETAILS OF PROJECT

*(Under the following heads on separate sheets)*

16. Introduction (not to exceed 2 pages or 1000 words)

Globally prostate cancer is the second most frequently diagnosed cancer and sixth leading cause of cancer death in men. There is an increasing trend in several metropolitan cities of India [1]. During treatment regime androgen-dependent tumours may relapse, finally giving rise to advanced disease characterized by loss of androgen dependence resistance to therapy. Incidence is increasing rapidly affecting men over 50 years old and an ideal candidate for chemoprevention [2]. Standard first line of treatment is castration; however this results in androgen synthesis from other tissues and over expression of androgen receptors [3]. Antiandrogen steroids like abiraterone are given along with prednisone [4].

Arachidonic acid is present in cell membranes as esters of phosphorylated triglycerides as phospholipids. In response to biological stimulus these arachidonic glycerides release free arachidonic acid by the action of lipases. The free arachidonic acid undergoes many transformations into several products regulated by two important pathways. One is cyclooxygenase and other is lipooxygenase pathway leads to transformation of arachidonic acid in to pro inflammatory prostaglandins and leukotrienes respectively. The expression of cyclooxygeneases and lipoxygenases are induced by several extra cellular signals including pro-inflammatory and growth-promoting stimuli. All signals converge to the activation of mitogen-activated protein kinases (MAPK) that regulate both at the transcriptional and post-transcriptional level through NFkB activation [5].

*Boswellia serrata* Roxb. (Burseraceae) oleo gum resin is a complex mixture containing series of mono,-sesqui,-di and triterpinoids. *Boswellia* resin and its individual components have been shown to be effective against rheumatoid arthritis, chronic colitis, ulcerative colitis, skin allergies and ulcers, peritumoral brain edema, osteoarthritis and inflammation [6-9]. In addition to anti-inflammatory activity, *in vitro* and *in vivo* research has shown that extract of *boswellia* species exert anticarcinogenic, antiproliferative, anti-tumor, apoptotic and cytostatic activity [10-12]. Triterpinoids are larger class of natural compounds that exhibit biologically and pharmacologically interesting properties [13]. BA has been shown to be effective anti inflammatory molecule and action is mediated through inhibition of NFkB, COX-2 and 5-LOX [14].

In prostate cancer cells there is sustained expression of Akt and loss of PTEN which is inhibitor of Akt activation. Therefore Akt signaling pathway plays a key role in the regulation of

cell division and survival of prostate cancer [15]. Many therapeutic strategies have been designed for inhibitors of Akt pathway as potential targets. Since Tirucallic acids are 5-LOX inhibitors and inhibit Akt signalling pathways few studies have shown that they are able to induce apoptosis in prostate cancer cells.

In present project we propose to study the molecular mechanism of modulating Akt and NFkB pathways using modified derivatives of tirucallic acids with relevance to prostate cancer. Such studies will help to understand various cellular processes involved in apoptosis will help in designing drugs both for androgen sensitive and insensitive prostate cancer cells.

16.1 Origin of the proposal

Triterpinoids a major class of natural compounds are biologically and pharmacologically active showing effect against rheumatoid arthritis, chronic colitis, ulcerative colitis, skin allergies, peritumoral brain edema, osteoarthritis and inflammation. *Boswellia serrata* Roxb. (Burseraceae) oleo gum resin is a complex mixture containing series of mono,-sesqui,-di and triterpinoids. In addition to anti-inflammatory activity, *in vitro* and *in vivo* research has shown that extract of *boswellia* species exert anticarcinogenic, antiproliferative, anti-tumor, apoptotic and cytostatic activity. We have standardized method as published earlier for isolating triterpenoids, Boswellic acids and Tirucallic acids. Using these pure forms of tirucallic acids we made few derivatives and analyzed for cytotoxic activity in various cancer cell lines. The Akt signalling pathway plays a key role in the regulation of cell division and survival of cancer cells. There is sustained expression of Akt in prostate cancer cells. Therefore inhibitors of Akt pathway can be potential therapeutic targets for prostate cancer. Studies on Tirucallic acid have shown that they are potential 5-LOX inhibitors and inhibited Akt signalling pathways inducing apoptosis in prostate cancer tumours. In present study we want study the effect of Tirucallic acid derivatives in modulating Akt pathway and regulation of apoptosis.

16.2 (a) Rationale of the study supported by cited literature

We isolated 3-oxo-tirucallic acid along with β-boswellic acids from *Boswellia serrata* gum (Shenvi and Reddy, 2013) in our earlier work on boswellic acids funded by DBT. From literature, we observed that tetracyclic triterpinoids and their structurally modified A-ring analogues exhibited good cytotoxicity. However many studies are reported on A-ring modification of terpenoids and steroids, β-boswellic acids and 3-oxo-tirucallic acid modification is not mentioned much in the literature. Hence we wanted to study the effect of A-ring modified

compounds on various cancer cell lines. Though, a study by Fu-Yue and Ren-Wang (2011) shows that three tetracyclic triterpinoids viz., 3-oxo-tirucallic acid, 3α-acetoxy-tirucallic acid and 3β-acetoxy-tirucallic acid could induce apoptosis in human prostate cancer, we want to study the mechanism of apoptosis and involvement of Akt signaling. Such detailed study on understanding molecular mechanism recruiting modified derivatives of Tirucallic acid will give insights to designing novel molecules for prostate cancer therapeutics.

(b) Hypothesis

Several triterpenoids, including ursolic and oleanolic acid, betulinic acid,celastrol, pristimerin, lupeol, and avicins possess antitumor and anti-inflammatory properties. In addition to anti-inflammatory activity, *in vitro* and *in vivo* studies have shown that extract of *boswellia* species exert anti-tumor, anticarcinogenic, antiproliferative, and apoptotic and cytostatic activity. Studies have shown A-ring modified tetracyclic triterpinoids showed significantly better cytotoxic activity than parent compound. We synthesized and studied the effect of A-ring modified 3-oxo tirucallic acids on various cancer cell lines in particular to prostate cancer cell line. We observed better antiproliferative activity with prostate cancer cell. Therefore we believe that understanding mechanism of molecular signaling pathway in apoptosis using modified derivatives of tirucallic acids will make way to design novel drug molecules to prostate cancer.

(c) Key questions.

1. Whether modified derivatives of 3-oxo tirucallic acid showing better cytotoxic activity can regulate Akt pathway?
2. Does it also affect other important cell signaling pathways like NFkB since these molecules are known to be Lipoxygenase inhibitors?
3. Can derivatives modulate cell signaling to induce apoptosis in both androgen sensitive and insensitive prostate cancer cells?

16.5 Current status of research and development in the subject (both international and national status)

**National Status**

Prostate cancer is a major public health burden worldwide with varied incidence in Asia. However there is consistent increase in most of the Asian countries last 25 years presumably reflecting shifts in diet and life style factors. In India first population based cancer registry was established in Mumbai (Bombay), by Indian Cancer Society in 1964 covering the urban

population of Greater Mumbai. NCRP was launched by ICMR in 1981, establishing another two population based cancer Registries at Chennai and Bangalore. Subsequently new population based cancer Registries were commissioned by ICMR under the network of NCRP at Bhopal and New Delhi in 1986. According to a study 'Trends in the Prostate Cancer Incidence in India' *Asian Pacific J Cancer Prev,* 9, 141-144 by Dr. Balakrishana. B. Yeole there is increase in rate of incidence of prostate cancer in India as well as in Asia. There has to be priority for preparations necessary for effective control of prostate cancer [16].

*Boswellia serrata* is a moderate to large sized branching tree growing in dry mountain parts of India. In ancient times, natural resins were used in cultural functions and have been used in folk medicine for centuries to treat chronic inflammatory disorders as well as quoted in many classical ayurvedic texts. It has been used as anti pyretic, anti inflammatory, analgesic, antiatherosclerotic and hepatoprotective. *In vitro* studies by Ammon et al [17] have shown inhibition of leukotriene synthesis through 5-LOX. Singh et al have shown anti-inflammatory activity of mixtures of boswellic acids in paw edema of rats and mice. Gayathri et al have reported inhibition of TNF-α, IL-1 and MAP kinases in human peripheral blood mononuclear cells [18]. Clinical trials have shown extracts are effective in osteoarthritis [19]. Pang X et al have shown that Acetyl-11- keto-β-boswellic acid inhibits prostate tumor growth by suppressing vascular endothelial growth factor receptor 2- mediated angiogenesis [20].

Many products of boswellic acids are manufactured like Shallaki® by Himalayan Drug Company, Niltan ® manufactured by Dr. Reddys and Rheumatic-X® manufactured by Sunrise Herbals.

Despite of its historical, cultural and medicinal importance Boswellia has been thoroughly studied there is still gap exist between our traditional knowledge and available scientific data [14]. In these directions we are attempting to use these novel molecules Tirucallic acids derived from Boswellia to study the molecular mechanism of prostate cancer.

**International status**

Prostate cancer is the most common cancer the second leading cause of death after lung cancer on both USA and Australia, and the third after lung and colo-rectal cancers in Europe. It is dependent on androgens primarily for development, growth and progression. Later it becomes hormone independent and can reoccur even after androgen deprivation therapy. The novel approach to drug discovery is ability to target both androgen-dependent and -independent cells in order to avoid reoccurrence considering multi targeted approach. Many studies have leads to understand the biological and molecular mechanisms driving prostate cancer development and

progression. It is more important to reveal molecular determinants involved in the process of cancer development and progression using novel molecules in order to find chemopreventive agents useful in targeted prevention agents that could be useful in targeted prevention and treatment strategies against prostate cancer. Cancer cells are known to alter multiple cell signaling pathways [21]. In prostate cancer the altered proteins are produced due to mutations or as a result of faulty communications in these cells. The important cellular signaling pathways in prostate cancer are androgen receptor (AR), Akt, nuclear factor-kB (NF-kB), Wnt, Hedgehog (Hh) and Notch pathways (Fig.2). The alteration in these pathways can occur at different stages of prostate cancer from early to advanced stage.

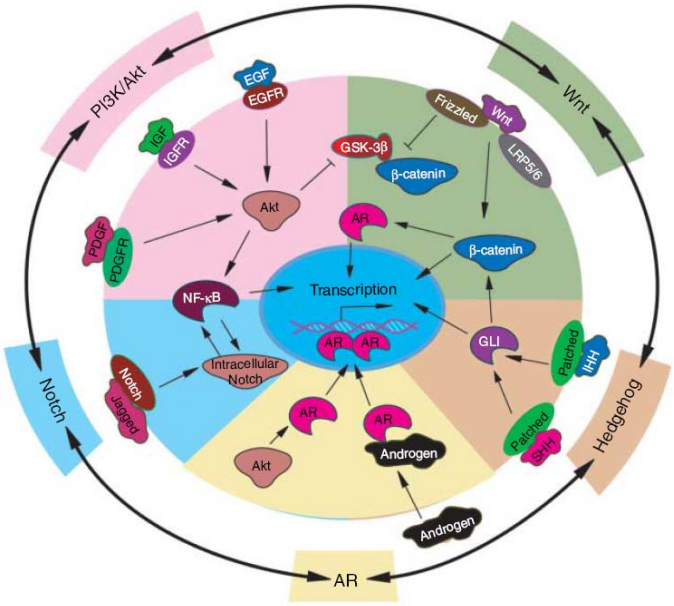


**Figure.2** Major Cell signaling pathways altered during the development and progression of prostate cancer. The crosstalk between AR, Akt, NF-kB, Wnt, Hedgehog, and Notch signaling plays critical roles in prostatic carcinogenesis. Therefore, targeting these signaling pathways is important strategy for the prevention of prostate cancer. {Adapted from Fazlul H Sarkar, Yiwei Li, Zhiwei Wang and Dejuan Kong 'Novel targets for prostate cancer chemoprevention Endocrine-Related Cancer' (2010) 17 195–212}.

Natural products from various sources have tested for prostate cancer related targets with promising results. Many such compounds are Jejimalide B, spisulosine, pristimerin, celastrol, withaferin A, and several pentacyclic triterpenoids such as betulinic, ursolic and boswellic acids (BA) shows promising future drug development. Pentacyclic triterpenoids among triterpenoids mentioned above are extensively found in fruits, vegetables and medicinal plants [22]. Boswellic acids triggered apoptosis and inhibited proliferation in prostate cancer cells probably by down regulating AR receptor that leads to decreased expression of vascular endothelial growth factor (VEGF) and survivin, and caspase-dependent PARP cleavage [23,24]. BA was shown to induce

proteasome-dependent degradation of Sp proteins in LNCaP cells [23, 25]. *In vivo*, BA (10 and 20 mg/kg/day) inhibited tumor growth in athymic nude mice bearing LNCaP cell xenografts, which was accompanied by decreased expression of Sp1, Sp3 and Sp4 proteins and VEGF, and increased apoptosis in tumors from BA-treated mice [23].

Apart from Boswellic acids, *Boswellia serrata* resin contains tetracyclic triterpenes called tirucallic acids. The major constituent is 3-oxo-tirucallic acid known to be 5-LOX inhibitor. In prostate cancer cell lines, 3-oxo-TA inhibited phosphorylation of Akt, GSK-3β and BAD following nuclear accumulation of β catenin and cmyc inducing apoptosis. In present study we want to use the derivatives of 3-oxo-TA which has already shown better activity in initial studies performed in our laboratory. We want to use these novel molecules to understand the molecular mechanisms of cell signaling pathways leading to proliferation and carcinogenesis of prostate cancer. Such studies will help to design therapies using innovative strategies targeting selective pathways.

**References**


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16.6 The relevance and expected outcome of the proposed study

The Project will be an important step in understanding the cell signaling pathway involved in pathogenesis of prostate cancer. However the mechanism is known for activation of NFkB leading to apoptosis, detailed investigation of molecular mechanism of Akt and NFkB pathway recruiting modified derivatives of 3-oxo-tirucallic acid may help in better understanding of inflammatory response. We also propose study detailed apoptotic mechanism linking to these cells signaling pathways will add value in developing novel therapeutic strategies for treating prostate cancer.

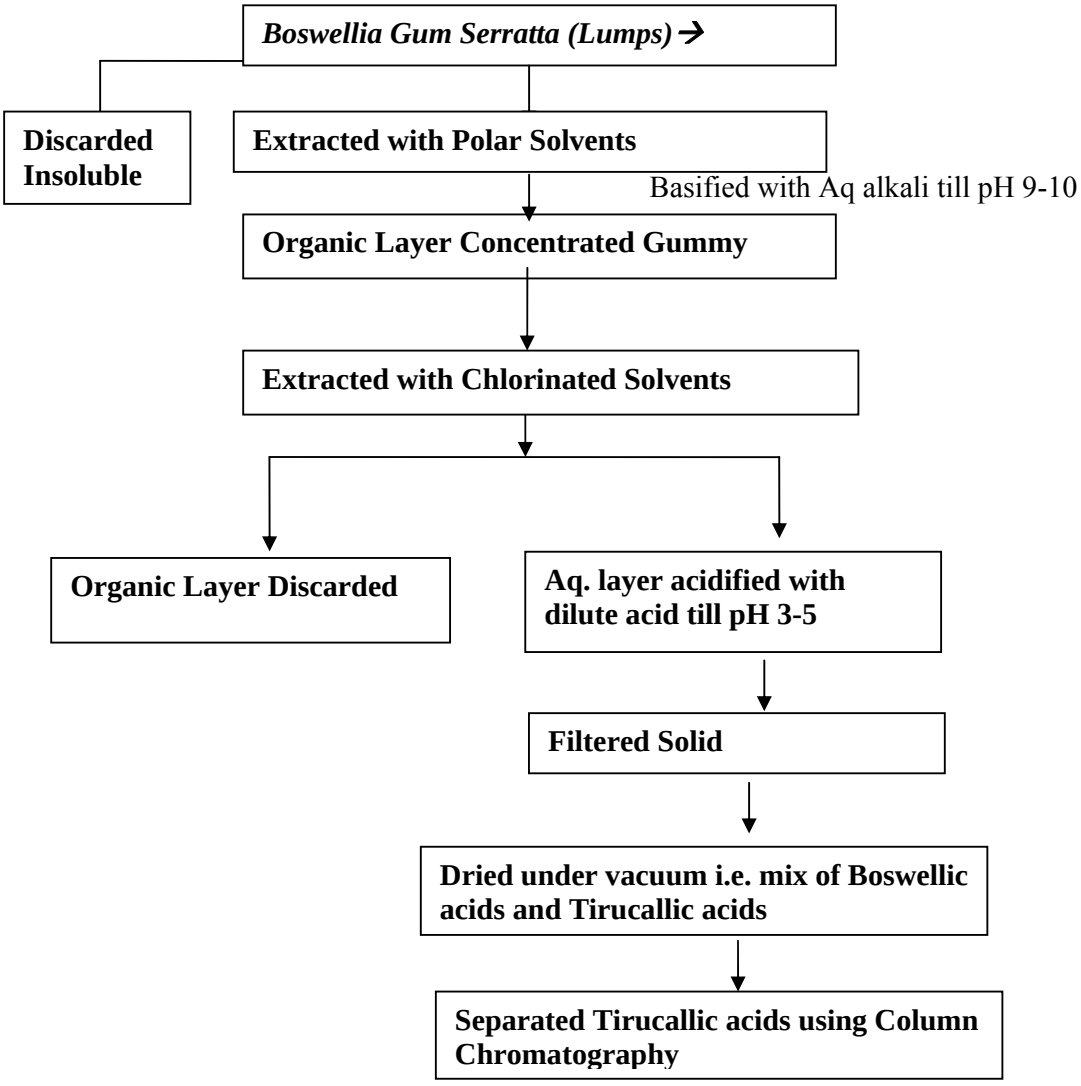
16.7 Preliminary work done so far

We have standardized method as published earlier for isolating triterpenoids, Boswellic acids

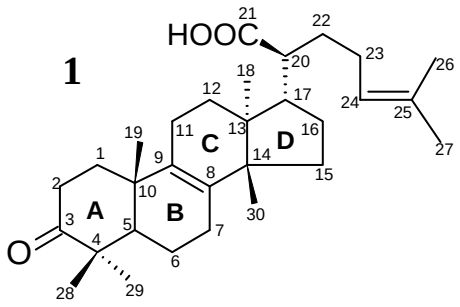
and Tirucallic acids. *Boswellia serrata* gum resin is separated into two fractions by solvent extraction and alkali treatment.

- An acid fraction
- A neutral fraction

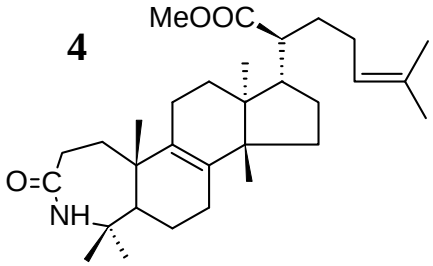
The total acid fraction is pharmacologically significant to give the therapeutic benefit. In our method if we use a 65-70% standardized organic acids raw material in the chromatographic method these gum resins subjected to repeated column chromatography over silica gel and eluted with organic solvent in different proportions furnishes individual component into pure forms. The flow diagram for isolation of Tirucallic Acids is depicted below.



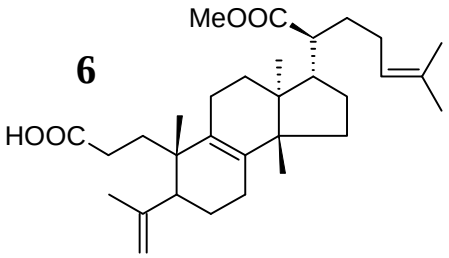
The above percentages of different fractions achieved in our experiments are comparable to reported percentages in literature. Using these pure forms of tirucallic acids (compound 1) we made few derivatives (compound 2-6) and analyzed for cytotoxic activity in various cancer cell lines namely HeLa (human cervical cancer), SW-982 (human synovial sarcoma), MCF-7 (human breast cancer), PC-3 (human prostate cancer) and IMR-132 (human neuroblastoma) by MTT assay using camptothecin as standard (Table.1).




*3-oxo-tirucall-8, 24-dien-21-oic acid (3-oxo-tirucallic acid,*




*Methyl-A-homo-4-aza-3-oxo-tirucall-8, 24, dien-3-one-21-oate*




*Methyl- 3-carboxylic, 3, 4-seco--tirucall-8, 24-dien-21-oate*

**Table 1.** Cytotoxic activity of A-ring modified analogues of *methyl 3-oxo-tirucall-8, 24-dien-21-oate* (**1-6**)

| Compounds | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|
| | HeLa | SW-982 | MCF-7 | PC-3 | IMR-32 |
| 1 | 103.18±6.39 | 83.38±13.64 | >150 | 140.42±15.88 | 81.22 ± 4.19 |
| 2 | 117.21±7.31 | 90.55±14.78 | >150 | 93.40±15.11 | >150 |
| 3 | 96.17±12.48 | 56.92±4.80 | 107.96±7.29 | 109.03±9.32 | 146.75 ± 15.56 |
| 4 | 69.54±4.98 | 54.51±13.97 | 77.44±8.71 | **37.12±10.01** | 57.50 ± 3.79 |
| 5 | >150 | 82.26±12.14 | >150 | 104.35±10.82 | >150 |
| 6 | 59.27±14.12 | 63.08±15.82 | >150 | **52.31±14.34** | 63.22 ± 3.20 |
| Camptothecin (Std) | 4.39± 0.44 | 28.64 ± 10.89 | 10.85 ± 0.39 | 4.82 ± 0.86 | 6.07± 2.31 |

Conversion of naturally occurring acid **1** into its methyl ester did not alter its anti-tumor activity, but A-ring modified derivatives showed good improvement over their parent compound (**4 and 6**). These compounds were further studied in prostate cancer cell line in which it showed good activity (Fig.3). PC-3 cells were treated with compound 4 and 6 at LD50 dose and we could see apoptotic cells within 12 hrs of treatment (Fig.4). Further these PC-3 cells were stained with propodium iodide and subjected to FACS in BD-calibur system for cell cycle analysis. We observed all the three compounds were able to induce apoptotic effects. However compound 4 and 6 were better than parent compound 1 inducing apoptosis at much smaller doses.

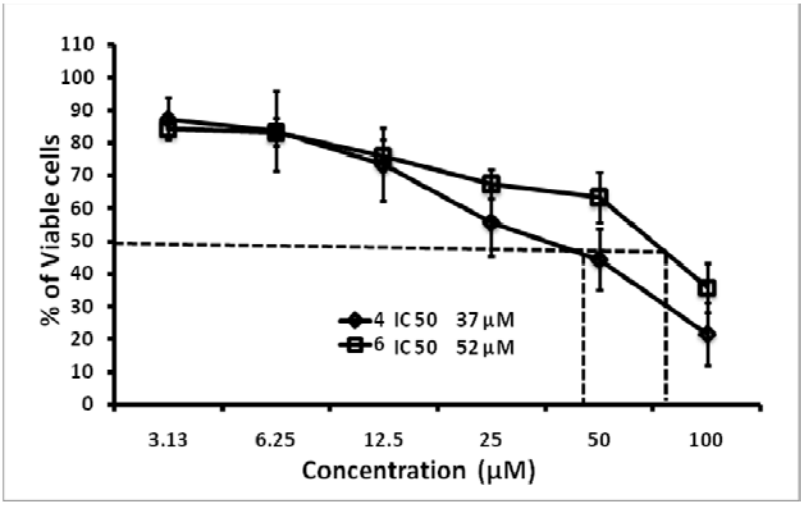



**Figure.3.** Dose-response curves of compound **4** and **6** showing viability/proliferation of PC-3 cells.

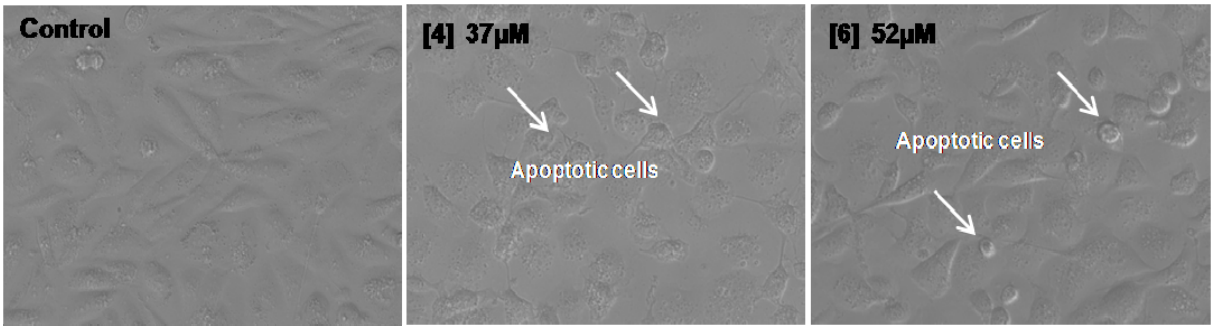



**Figure.4.** Compounds 4 and **6** inducing apoptotic cell death after 24 h of incubation with PC-3 cells at 20X magnification

**Publication:** Beckmann rearrangement products of methyl 3-oxo-tirucall-8, 24-dien-21-oate and their anti-tumor activity, Suvarna Shenvi, K. Rijesh, **Latha Diwakar**, G.Chandrasekara Reddy accepted in Phytochemistry letters.

17. Specific objectives (should be written in bulleted form, a short paragraph indicating the methods to be followed for achieving the objective and verifiable indicators of progress should follow each specific objective).

1. ***Effect of derivatives of 3-oxo-TA on NFkB pathway leading to expression of 5-LOX and Cox-2 expression.***

2. ***Anticancer effect of 3-oxo-TA derivatives on androgen insensitive (LNCaP) and androgen sensitive (PC-3) cells culminating them to apoptosis.***
3. ***Expression of different apoptotic markers, including changes in mitochondrial membrane potential.***
4. ***To evaluate anticancer potency of derivatives in few other cancer cell lines and toxicological effect on normal cells.***

18. Work Plan: should not exceed 3-4 pages (the section can be divided according to the specific aims and under each specific aim, the following should be stated clearly as sub headings)

18.1 Work plan (methodology/experimental design to accomplish the stated aim)

1. *Effect of derivatives of 3-oxo-TA on Akt and NFkB pathway leading to expression of 5-LOX and Cox-2 expression.*
   **Methodology:** Prostate cancer cells will be pretreated with effective concentration of derivatives of 3-oxo-TA followed by exposure to IL-1 or TNF-α. Inhibition in NFkB activation and translocation, further expected decrease in MAPK induced IKK phosphorylation can be determined by western blot. Since 3-oxo-TA is 5-LOX inhibitor we want to study how it is going to regulate the expression 5-LOX and Cox-2 through NFkB pathway by western blot.

2. *Anticancer effect of 3-oxo-TA derivatives on androgen insensitive (LNCaP) and androgen sensitive (PC-3) cells culminating them to apoptosis.*
   **Methodology:** 3-oxo-TA is known to induce apoptosis by inhibiting Akt. In present study we will treat prostate cancer cells with derivatives of 3-oxo-TA following with or without stimulating with IL-1 or TNF-α and observe translocation of transcription factors like c-myc and β-catenin which are regulated by Akt pathway. We would also want to study apoptotic effect of 3-oxo-TA derivatives by release of Cytochrome C, Apaf-1 and Bcl/Bax ratio. Finally the expression of caspase-3 in inducing apoptosis can be studied by western blot.

3. *Expression of different apoptotic markers, including changes in mitochondrial membrane potential.*
   **Methodology:** Changes in mitochondrial potential can be determined by using mitotracker dye/ JC-1 after treating cells with IL-1 or TNF-α. Cytoplasmic and mitochondrial fractions will be prepared from prostate cancer cells stimulated with IL-1 or TNF release of cytochrome C and Apaf-1 is observed by western blot. Bcl/Bax ratio can be determined by western blot/immunoflorescence in same samples. Expression of caspase-3 final effector caspase in inducing apoptosis can be observed by western blot.

4. *To evaluate anticancer potency of derivatives in few other cancer cell lines and toxicological effect on normal cells.*
   **Methodology:** Tirucallic acid derivatives can be evaluated for on different cancer cell line and normal cells can be evaluated by MTT assay. Further anticancer potency can be

evaluated in FACS by propidium iodide staining for cell cycle and Annexin V staining for apoptotic activity.

18.2 Connectivity of the participating institutions and investigators (in case of multi- institutional projects only)

**-Not applicable-**

18.3 Alternate strategies (if the proposed experimental design or method does not work what is the alternate strategy)

Experimental strategies proposed in the methodology are standardized methods so alternate strategies are not required

19. Timelines: (Please provide quantifiable outputs)

| Period of study | Achievable targets |
|---|---|
| 6 Months | Cancer cells will be stimulated with cytokine at different time points to study the translocation of NFkB from cytosol to nucleus. Once the time points are established expression of proinflammatory enzymes cyclooxygenase-2 and 5-lipooxygenase was determined by western blot. |
| 12 Months | Changes in mitochondrial potential after cytokine treatment in prostate cancer cells can be determined by JC-1/Mitotracker dye. Cytosolic and Mitochondrial fractions will be prepared from cell treated with cytokine to observe release of cytchrome c and Apaf-1. |
| 18 Months | Bcl/Bax ratio will be measured by western blot/ immunoflorescence in cells treated with Tirucallic acid derivatives. Expression of caspase-3 final effector caspase in inducing apoptosis can be observed by western blot. |
| 24 Months | Expected decrease in MAPK induced IKK phosphorylation leading to NFkB activation and translocation after exposure to IL-1 or TNF-α and pretreatment with derivatives can be determined by western blot. Since 3-oxo-TA is 5-LOX inhibitor the regulation in the expression 5-LOX and Cox-2 can be observed by immunohistochemistry. |
| 30 Months | Prostate cancer cells will be treated with derivatives of 3-oxo-TA following with or without stimulating with IL-1 or TNF-α and observe translocation of transcription factors like c-myc and β-catenin by immunohistochemistry. Further to study apoptotic effect of 3-oxo-TA derivatives, release of Cytochrome C, Apaf-1 and Bcl/Bax ratio will be observed through western blot. Finally the expression of caspase-3 in inducing apoptosis can be studied by western blot. |

| | |
|---|---|
| 36 Months | Different cancer cell line and normal cells will be evaluated for toxicity of tirucallic acid derivatives by MTT assay. Further anticancer potency can be evaluated in FACS by propidium iodide staining for cell cycle and Annexin V staining for apoptotic activity |

20. Name and address of 5 experts in the field

| Sr.No. | Name | Designation | Address |
|---|---|---|---|
| 1 | Prof. RitaChristopher | Professor & Head | Dept. of Neurochemistry<br>NIMHANS<br>Hosur Road.<br>Bangalore-560 026.<br>Karnataka, India.<br>rita@nimhans.kar.nic.in |
| 2 | Prof. D. Loganathan | Professor | Department of Chemistry<br>Indian Institute of Technology Madras<br>Chennai-600036, Tamil Nadu |
| 3 | Dr. Siva Umapathy | Professor | Department of Inorganic & Physical Chemistry<br>IISc, Bangalore-560012<br>Email:<br>umapathy@ipc.iisc.ernet.in |
| 4 | Dr. B.V.Subba Reddy | Sr. Principal Scientist | Natural Products Chemistry<br>Indian Institute of Chemical Technology<br>Hyderabad-50007-Telangana<br>INDIA<br>Tel: +91-40-27193535<br>E-mail:<br>basireddy@iict.res.in |
| 5 | Dr. Ch. Prasada Rao | Professor | Department of chemistry<br>Osmania University<br>Hyderabad-500007-Telangana<br>INDIA<br>Tel: +91-9550506096<br>E-mail:<br>prasadraoch@yahoo.com |

## PART IV: BUDGET PARTICULARS

**Budget (In Rupees)**

**A. Non-Recurring (e.g. equipments, accessories, etc.)**

| S. No. | Item | Year 1 | Year 2 | Year 3 | Total |
|---|---|---|---|---|---|
| 1 | Fluorescence Microscope | 25,00000 | | | 25,00000 |

**Sub-Total (A) = 25, 00000**

**B. Recurring**

**B.1 Manpower (See guidelines at Annexure-III)**

| S. No. | Position No. | Consolidated Emolument | Year 1 | Year 2 | Year 3 | Total |
|---|---|---|---|---|---|---|
| 1 | Senior Research Fellow | 14000+HRA | 218400 | 218400 | 218400 | 655200 |
| 2 | Junior Research Fellow | 12000+ HRA | 187200 | 187200 | 187200 | 561600 |
| 3 | Lab assistant | 8000 | 96000 | 96000 | 96000 | 288000 |

**Sub-Total (B.1) = 1504800**

**B.2 Consumables**

| S. No. | Item | Quantity | Year 1 | Year 2 | Year 3 | Total |
|---|---|---|---|---|---|---|
| 1 | Media, serum and other consumable/ chemicals | As required | 3,00,000 | 3,00,000 | 3,00,000 | 9,00,000 |
| 2 | Phosphorylated antibodies for western blot | 2 vials each | 2,00,000 | 2,00,000 | 2,00,000 | 6,00,000 |
| 3 | Antibodies for immunohistochemistry | 2 vials each | 2,00,000 | 2,00,000 | 2,00,000 | 6,00,000 |
| 4 | Disposable Plastic ware and glassware | As required | 3,00,000 | 3,00,000 | 3,00,000 | 9,00,000 |

**Sub-Total (B.2) = 30, 00000**

| **Other items** | Consolidated Emolument | Year 1 | Year 2 | Year 3 | Total |
|---|---|---|---|---|---|
| **B.3 Travel** | | 50000 | 50000 | 50000 | 150000 |
| **B.4 Contingency** | | 200000 | 200000 | 200000 | 600000 |

| | | | | | |
|---|---|---|---|---|---|
| **B.5 Overhead (If applicable)** | | - | - | - | - |
| **Sub-total of B (B.1+B.2+B.3+B.4+B.5)** | | 1751600 | 1751600 | 1751600 | 5254800 |
| **Grand Total (A + B)** | | 4251600 | | | 7754800 |

**Note: Please give justification for each head and sub-head separately mentioned in the above table**.

**Justification A:**

Fluorescent Microscope is needed with image analyzing software as project involves lot of Immunohistochemistry work to study the expression of Cox-2, 5-Lox and translocation of some of the marker like cmyc and β-catenin. Fluorescence is also required to study the miotochondrial membrane potential using mitotracker dye.

**Justification B1:**

Project involves cell culture work and routine sterile condition hence JRF is required to maintain cell line and treatments. The techniques like immunohistochemistry needs skilled person hence one SRF has been requested will be involved in critical skills like molecular techniques and analytical techniques to execute the project on time. Lab assistant is required to keep up the sterile conditions and autoclaving etc.

**Justification B2:**

The Pure cytokine used to give inflammatory insult is very costly and antibody are very are essential as they are the indicators of apoptosis at different stages. Primary and secondary antibodies for detection in western blots are expensive. Phosphorylated antibodies needed to study cell signaling pathways are costly. The molecular biology reagents, antibodies and enzymes for PCR are expensive hence more budget for consumables are needed.

**Justification B3:**

The travel budget is mainly meant for some advanced training in some Centers of Excellence in cancer biology and for attending National level meetings in the area of cancer biology and natural product chemistry.

## PART V: EXISTING FACILITIES

**Resources and additional information**

The Vittal Mallya Scientific Research Foundation (VMSRF), a non-profit research organization, was established by The UB Group in 1987, in memory of late Shri. Vittal Mallya, the dynamic and illustrious founder of the group, as an independent centre for applied research with biotechnology as its main thrust. VMSRF is recognized by the Departments of Scientific &

Industrial Research (DSIR), Department of Biotechnology (DBT), and Council for Scientific & Industrial Research (CSIR) and The Ministry of Finance, Govt. of India. A governing body consisting of eminent personalities from industry & science oversee the functioning of the foundation. In a short span of time the centre has developed several novel technologies and products in the domains of health care & agriculture, with strong IPR fortification.

VMSRF has successfully completed 12 externally funded projects and 9 are ongoing projects funded by DBT, CSIR, DST, ICAR and IFS, Sewden which includes (major) Anticancer activity of Flavonoids, anti arthritic and anti-inflammatory molecules from Natural products, These molecules are being evaluated in animal models. Modification of oil composition in Jatropha by Metabolic Engineering; Increasing oil content in Safflower by Metabolic Engineering; Metabolic profiling in Tomato; Biosurfactants; Screening of plant based Antimicrobial and Anti-insecticidal drugs; Development of Botanical pesticide database; VMSRF has a strong work ethics and in-house skill sets in the field of Plant Molecular Biology; Natural Product Chemistry; Nutraceuticals; and Bioinformatics. In addition, various eminent scientists from reputed institutes across India are involved with VMSRF as collaborators. VMSRF has about 20 national and global patents to its credit as well and have quite a few technology transfers to its credit.

**b. Equipments**

| Sl. No. | Name of equipment | Make | Funding agency | Year of Procurement |
|---|---|---|---|---|
| 1 | Maxi cold | Pharmacia | VMSRF | 1991 |
| 2 | Rotavapour | Buchii | VMSRF/DBT | 1995 |
| 3 | $CO_2$ Incubator | Nuair | DBT | 1998 |
| 4 | Fluid bed dryer | Allience | VMSRF | 1998 |
| 5 | Tubular centrifuge | CEPA | VMSRF | 1999 |
| 6 | Biospectro Photometer | Varian | VMSRF | 1999 |
| 7 | Incubator shaker | NBS | VMSRF | 2000 |
| 8 | Elisa Reader | Softmax | DBT | 2000 |
| 9 | Spectrophotometer | Varian | VMSRF | 2000 |

| | | | | |
|---|---|---|---|---|
| 10 | HPLC | Shimadzu | VMSRF | 2003 |
| 11 | Laminar flow hoods | Alpa | VMSRF | 2004/2006 |
| 12 | Gel documentation | UVI Tech | DBT | 2006 |
| 13 | GC MS | Shimadzu | VMSRF | 2006 |
| 14 | Milli Q system | Millipore | DBT | 2006 |
| 15 | Spray Drier | Pawan Engineering | VMSRF | 2007 |
| 16 | Micro balance | Sartorius | VMSRF | 2007 |
| 17 | GC FID | Shimadzu | VMSRF | 2007 |
| 18 | Light Microscope | Olympus | VMSRF | 2008 |
| 19 | Preparative HPLC | Waters | VMSRF | 2000 |

1. Other resources such as clinical material, animal house facility, glass house. Experimental garden, pilot plant facility etc.

**Animal House Facility**

Animal House to ensure humane care to the animals and their use, necessary for the pursuit and achievement of scientific excellence and is build and maintained strictly in accordance to the CPCSEA guidelines and Institutional Animal Ethics Committee. Different species and strains are kept separately in animal rooms maintained under standard room temperature of $22^{o}$ to $25^{o}$ C, 50-55% relative humidity, 12:12 hour light and dark cycle with 100% fresh air circulation and uninterrupted power and water supply. Animal house facility is looked after by a full time well qualified Veterinary officer led by a team of technical assistants and animal caretakers well experienced and trained in animal care, breeding and husbandry. All the procedures are followed in order to keep the animal house hygienic and infection free is routinely followed strictly by the staff under the guidance of Veterinary officer.

## PART VI: DECLARATION/CERTIFICATION

It is certified that

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) the same project proposal has not been submitted to any other agency for financial support.

c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)

d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.

e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.

f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.

h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.

j) the Institute assumes to undertake the financial and other management responsibilities of the project.

**Signature of Executive Authority**
**of Institute/University with seal**
**Date:**

**Signature of Principal Investigator**
**Date :**

**Signature of Co-Investigator**
**Date:**

**Signature of Co-Investigator**
**Date:**

**PART VII: PROFORMA FOR BIOGRAPHICAL SKETCH OF INVESTIGATORS**

Provide the following information for the key personnel in the order listed on PART II.
Follow this format for each person. **DO NOT EXCEED THREE PAGES**

**Principal Investigator**

**Name:** Dr. Latha Diwakar

**Date of Birth:** 30-11-1973 **Sex: (M/F):** Female

**Designation**: Research Scientist **Department**: Biology

**Institute/University:** Vittal Mallya Scientific Research Foundation

**Address:** #94/3&94/5, 23rd Cross, 29th Main, BTM II Stage, Bangalore – 560 076

**Telephone**: 080-26687216, 26687223 Telex: **Fax:** 080-26687170

**Email**: latha@vmsrf.org

**No. of Projects being handled at present**: one

**Education (Post-Graduation onwards & Professional Career)**

| Sl No. | Institution Place | Degree Awarded | Year | Field of Study |
|---|---|---|---|---|
| 1 | National Brain Research Center | Ph.D | 2004 | Neuroscience SRF, National Brain Research Center |
| 2 | Mysore University | M.Sc. | 1996 | |

**Position and Employment** (Starting with the most recent employment)

| Sl No. | Institution/Place | Position |
|---|---|---|
| 1 | Vittal Mallya Scientific Research Foundation | Scientist |
| 2 | National Institute of Mental Health & Neurosciences | Postdoctoral Fellow |
| 3 | National Brain Research Center, Gurgaon & Indian Institute of Science, Bangalore | Senior Research Fellow |
| 4 | National Institute of Mental Health & Neurosciences | Junior Research Fellow |
| 5 | Adichunchanagiri Cancer Research Center | Junior Research Fellow |
| 6 | Central Food Technological Research Institute, Mysore | Project Assistant |

**Honors/Awards**

**DST- Fast track grant**

- Awarded Travel grant to attend International Brain Research Organization School at Hong Kong - 2006.
- Awarded Travel grant to attend Neurodegeneration workshop at Melbourne, Australia - 2007.

**Professional Experience and Training relevant to the Project**

**Dr. Latha Diwakar** has experience in cell biology, drug metabolism and enzyme kinetics at NBRC, Gurgaon, IISc and NIMHANS at Bangalore. She has worked on animal model of Parkinson's, motor neuron disease and ischemia. She has also experience in molecular techniques like transfection and transformation. She has got a paper in Faseb journal on role of ASK (Apoptosis Signaling Kinase) in animal model of Parkinson's. The project on antioxidant and anticancerous effect of flavonoids has been recommended for financial support from DST from young scientist scheme.

**Publications** (Numbers only).

**Books:** .Nil **Research Papers**: 12 **Reports:** Nil **General articles:** Nil

**Patents:** Nil **Others (Please specify):** Nil

**List maximum of five recent publications relevant to the proposed area of work.**


1. Shenvi S, Kumar K, Hatti KS, Rijesh K, **Diwakar L,** Reddy G.C. (2013), Synthesis, anticancer and antioxidant activities of 2,4,5-trimethoxy chalcones and analogues from asaronaldehyde: Sturcture-activity relationship. European Journal of Medicinal Chemistry 62 (2013) 435e442.
2. Hatti KS, **Diwakar L**, Rao GV, Kush A, Reddy GC 2009 Abssiyone and related flavonoids as potential steriodogenesis modulators. Bioinformation.
3. **Diwakar L**, Ray A, Ravindranath V, 2008. Complex I assay in mitochondrial preparations from CNS. Curr.Protoc. Toxicol 38:17.10. 1-17.
4. **Diwakar L**, Kenchappa RS, Annepu J, Ravindranath V, 2007 Downregulation of Glutaredoxin but not glutathione loss leads to mitochondrial dysfunction in female mice CNS: Implications in excitotoxicity. Neurochem Int.51, 37-46.
5. Karunakaran S, **Diwakar L**, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, and Ravindranath V. 2007 Activation of apoptosis signal regulating kinase 1(ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: protection by alpha-lipoic acid. FASEB J. 21:2226-36. (Co- first author).

**Research Support**

**Ongoing Research Projects**

| Sl No. | Title of Project | Funding Agency | Amount | Date of sanction and Duration |
|---|---|---|---|---|
| 1 | "Chemically synthesized derivatives of Boswellic Acid as modulators of cytokine mediated cell signaling: implications in chronic inflammatory diseases" | DBT | 35.7 lakhs | Dec 2010 – Dec 2013 |
| 2 | Effect of fermented papaya preparation in modulating neuroinflammation: implications in neurodegeneration | DBT | 37.824 lakhs | April 2013- March 2016 |

**Completed Research Projects** (State only major projects of last 3 years)

| Sl No. | Title of Project | Funding Agency | Amount | Date of completion |
|---|---|---|---|---|
| 1 | **"**Deciphering cytotoxic, antioxidant and anti cancerous activity of chemically synthesized prenylated and non prenylated flavonoids". | Department of Science and Technology-Fast Track | Rs.19.00 lakhs | October 2013 |

**Place:** Bangalore
**Date:**

**Signature of Investigator**
**(Latha Diwakar)**

**Co-Investigator**

**Name:** Dr. Venkatesham Uppala

**Date of Birth:** 15/06/1972 **Sex (M/F):** M

**Designation:** Research Scientist **Department:** Chemistry

**Institute/University:** Vittal Mallya Scientific Research Foundation

**Address:** #94/3 & 94/5, 23rd cross, 29th Main, BTM II stage, Bangalore, Karnataka.

**Pin :** 560076 **Phone:** 08026687223 **Email:** venkatesham@vmsrf.org

**Number of Research projects being handled at present:** 2

**II) Education Details:**

| Sl. No. | Institution / Place | Degree Awarded | Year | Field of Study |
|---|---|---|---|---|
| **1** | Bhiknur PG college, Osmania University | M Sc | 1995 | Organic Chemistry |
| **2** | Indian Institute of Chemical Technology/ Hyderabad | Ph D | 2002 | Organic chemistry |

**III) Employment Details:**

| Sl. No. | Institution / Place | Position | From (Date) | To (Date) |
|---|---|---|---|---|
| 1 | University of Saskatchewan/Canada | Research Fellow | 14/02/2001 | 30/11/2002 |
| 2 | National Taiwan University/ Taiwan | Post Doctoral Fellow | 04/02/2003 | 31/07/2004 |
| 3 | National Taiwan Normal University/ Taiwan. | Post Doctoral Fellow | 01/08/2004 | 31/07/2006 |
| 4 | Chembiotek/ TCG Life Sciences PVT LTD./ Kolkata | Senior Research Scientist | 19/08/2006 | 30/10/2008 |
| 5 | Connexios Life Sciences PVT LTD/Bangalore | Senior Scientist | 17/11/2008 | 31/07/2013 |
| 6 | Creative Organics/ Bangalore | R & D Manager | 30/08/2013 | 30/05/2014 |
| **7** | Vittal Mallya Scientific Research Foundation/Bangalore-560076 | Research Scientist | 16/06/2014 | Till date |

**IV) Honors/Awards:**

| | No. | Description |
|---|---|---|
| **A) International** | 2 | 2003 to 2006 awarded Post Doctoral Fellowship from National Science Council, Taiwan.<br>2001 to 2002 awarded Research Fellowship from University of Saskatchewan, CANADA. |
| **B) National** | 3 | 1996 awarded Junior Research Fellowship from Council of Scientific and Industrial Research (CSIR), New Delhi, INDIA.<br>1996 awarded Graduate Aptitude Test in Engineering (GATE) by University Grants Commission (UGC), New Delhi, INDIA.<br>1995 awarded National Eligibility Test (NET) in Masters Level from the Andhra Pradesh College Service Commission; Hyderabad (A. P), INDIA; accredited by University of Grants Commission (UGC). |

**V) Publications: International – 16 National – 4**

**List of publications**

1. CYCLIC AMIDE DERIVATIVES AND USES THERE OF. Jagannath Madanahalli Ranganath RAO,Uppala VENKATESHAM, Jenson George, George FERNAND, Sivanageswara Rao DOPPALAPUDI, G R Madhavan, Nagarajan Arumugam, Mohammed Ansari, K Murugavel, Jidugu PRADEEP, Sulthan ALLAVUDDEEN,K Vijayaramalingam, Hampelingaiah Shiva PRASAD, Augustine Michael RAJ, S Gnanavel, Ramamoorthy Kottamalai,Naresh M P S BABU, Bommegowda Yadaganahalli KENCHEGOWDA. WO/2013/128465A1 2013.

2. On the scope of diastereoselective aziridination of various chiral auxiliaries derived N- and Oenones with Naminophthalimide in the presence of lead tetraacetate. Pei-Wen Duan, Ching-Chen Chiu, Wei-Der Lee, Li Shiue Pan, Uppala Venkatesham, Zheng-Hao Tzeng, Kwunmin Chen. Tetrahedron: Asymmetry, 2008.

3. Preparation of secolycorines against acetylcholinesterase. S.-S. Lee, U.Venkatesham, Ch. Prasad Rao,S.-H. Lam, J.-H. Lin. Bioorganic & Medicinal Chemistry. 2007.

4. Diastereoselective allylation of ?- ketoamides bearing camphor Ntosylpyrazolidinone auxiliary: efficient synthesis of highly optically active two stereoisomers. J.-H. Chen, U. Venkatesham, L.- C. Lee, K. Chen. Tetrahedron, 2006.

5. Highly diastereoselective allylation and reduction of chiral camphor derived a-ketoamides. N. A. Kulkarni, S.-G. Wang, L.-C. Lee, H. R. Tsai, U. Venkatesham, K. Chen. Tetrahedron: Asymmetry. 2006.

**VI) Professional Experience and Training relevant to the Project**

Dr. Uppala has research experience in synthetic organic chemistry, medicinal chemistry, asymmetric synthesis, biotransformations and natural products chemistry developed preclinical candidates.

**Place:** Bangalore **Signature of Investigator**

**Date:** **(Venkatesham Uppala)**

**Co-Investigator**

**Name:** Dr. Anil Kush

**Date of Birth:** September 13, 1956 **Sex: (M/F):** M

**Designation**: Research Director **Department**: Plant biotechnology/ Biotechnology

**Institute/University:** Vittal Mallya Scientific Research Foundation

**Address:** # 94/3 & 94/5, 23rd Cross, 29th Main, BTM II Stage, Bangalore–560 076

**Telephone**: 080-26687216, 26687223 Telex: **Fax:** 080-26687170

**Email**: anil.kush@gmail.com

**No. of Projects being handled at present**: one

**Education (Post graduation onwards & Professional Career)**

| Sl. No. | Institutional Name | Degree Awarded | Year | Award/Prize/Certificate |
|---|---|---|---|---|
| 1 | Manipal University | MBA | 2004 | First Division |
| 2 | Paris University (Pasteur Institute) | Ph.D | 1986 | Tres Honorable |

**Research Experience in various Institutions (if necessary, attach separate sheets)**

- Post Doctoral Research Fellow at the Rockefeller University, NY, USA
- Post Doctoral Research Associate, Harvard University, MA, USA
- Principal investigator, Institute of Molecular and Cell Biology (IMCB), Singapore
- Director, (Biotechnology), Indo-American Hybrid seeds, Bangalore
- Executive Vice president, Reliance Life Sciences, Mumbai
- Director, Vittal Mallya Scientific Research Foundation, Bangalore

**Publications:**

Books: 3 (Chapters); Research Papers & Reports: 50 General Articles: 5
Patents: 8 Others: Nil

**List maximum of five recent publications relevant to the proposed area of work.**


1. Raghava T., Ravikumar P., Hegde R. and Kush A (2010) Spatial and temporal secondary metabolite response of tomato cultivars to herbivory and mechanical injury. Plant Science, Plant Sci., 179(5):520-6.
2. Chandregowda V, Kush A, Reddy GC (2009) Synthesis and invitro antitumor activities of novel 4-anilinoquinazoline derivatives. European Journal of Medicinal Chemistry 44, 3053-3062.

3. Chandregowda V, Kush A, Reddy GC (2009) Synthesis of benzamide derivatives of anacardic acid and their cytotoxic activity. European Journal of Medicinal Chemistry 44, 2711-2719.
4. Kaushik H S , Chandregowda V, Venkateswara RG, Kush A, Reddy G C (2009) In-silico Interaction Studies of Quinazoline Derivatives for their Inhibitory Action on Both Wild and Mutant EGFRs. J Proteomics Bioinform 2: 126-130.
5. Kaushik H S , Diwakar L, Rao G V , Kush A , Reddy GC (2009) Aromatase Abyssinones and related flavonoids as potential steroidogenesis modulators. Bioinformation 3(9):399-402.

**Ongoing Research Projects**

| Sl. No. | Title of Project | Funding Agency | Duration and Reference Number |
|---|---|---|---|
| 1 | Metabolic engineering of oil biosynthetic pathway in safflower [*Carthamus tintorius*] for fortification with Omega 3 FA. | DBT | February 2012 to January 2015 DBT/PR4077/AGR/02/832/2011 |

**Highlights of progress of the project(s) to date (in 200 words) for ongoing projects only**

Both projects are on metabolomics and genetic engineering of important plant systems. In the Jatropha Project, it is being tried to genetically modify the fatty acid biosynthesis mechanism to produce the seed oil which can be more efficiently used in automobile engines. The proof of concept of the genetic assembly has been developed in *Arabidopsis thaliana* and transformations on *Jatropha* are underway.

**Place:** Bangalore

**Date:**

**Signature of Investigator**
**(Dr. Anil Kush)**