Nimbolide is a terpenoid lactone derived from Azadirachta indica (Neem) that displays a variety of biological activities including anti-malarial and anticancer activity

1. Nimbolide is a more potent antiproliferative and apoptosis inducing agent and offers promise as a candidate agent in multitargeted prevention and treatment of cancer.
2. Nimbolide can sensitize tumor cells to chemotherapeutic agents through interaction with IKK, leading to inhibition of NF-κB-regulated proteins.
3. Nimbolide inhibits invasion and migration, and down-regulates uPAR chemokine gene expression, in two breast cancer cell lines.
4. Nimbolide attenuates the lipid accumulation, oxidative stress and antioxidant in primary hepatocytes.
5. Nimbolide has antibacterial potential.

References Information for Nimbolide:

Nimbolide reduces CD44 positive cell population and induces mitochondrial apoptosis in pancreatic cancer cells
Cancer Letters; ***vol.*** *413; (2018); p. 82 – 93*

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is highly aggressive disease and current treatment regimens fail to effectively curePDAC. Development of resistance to current therapy is one of the key reasons for this outcome. Nimbolide (NL), a triterpenoid obtained fromAzadirachta indica, exhibits anticancer properties in various cancer including PDAC cells. However, the underlying mechanism of this anticanceragent in PDAC cells remains undefined. We show that NL exerts a higher level of apoptotic cell death compared to the first-line agentgemcitabine for PDAC, as well as other anticancer agents including sorafenib and curcumin. The anticancer efficacy of NL was further evidencedby a reduction in the CD44+ as well as cancer stem-like cell (CSC) population, as it causes decreased sphere formation. Mechanistically,the anticancer efficacy of NL associates with reduced mutant p53 as well as increased mitochondrial activity in the form of increased mitochondrialreactive oxygen species and mitochondrial mass. Together, this study highlights the therapeutic potential of NL in mutant p53 expressing

Molecular docking, QSAR and ADMET based mining of natural compounds against prime targets of HIV
Journal of Biomolecular Structure and Dynamics; (2018); p. 1 – 16

Abstract: AIDS is one of the multifaceted diseases and this underlying complexity hampers its complete cure. The toxicity of existing drugsand emergence of multidrug-resistant virus makes the treatment worse. Development of effective, safe and low-cost anti-HIV drugs is amongthe top global priority. Exploration of natural resources may give ray of hope to develop new anti-HIV leads. Among the various therapeutictargets for HIV treatment, reverse transcriptase, protease, integrase, GP120, and ribonuclease are the prime focus. In the present study, we predictedpotential plant-derived natural molecules for HIV treatment using computational approach, i.e. molecular docking, quantitative structureactivity relationship (QSAR), and ADMET studies. Receptor-ligand binding studies were performed using three different software for preciseprediction – Discovery studio 4.0, Schrodinger and Molegrow virtual docker. Docking

scores revealed that Mulberrosides, Anolignans, Curcuminand Chebulic acid are promising candidates that bind with multi targets of HIV, while Neo-andrographolide, Nimbolide and Punigluconinwere target-specific candidates. Subsequently, QSAR was performed using biologically proved compounds which predicted the biological activityof compounds. We identified Anolignans, Curcumin, Mulberrosides, Chebulic acid and Neo-andrographolide as potential natural moleculesfor HIV treatment from results of molecular docking and 3D-QSAR. In silico ADMET studies showed drug-likeness of these lead molecules. Structure similarities of identified lead molecules were compared with identified marketed drugs by superimposing both the molecules.Using in silico studies, we have identified few best fit molecules of natural origin against identified targets which may give new drugs to combatHIV infection after wet lab validation.

Epoxyazadiradione suppresses breast tumor growth through mitochondrial depolarization and caspase-dependent apoptosis by targeting PI3K/Akt pathway
BMC Cancer; ***vol.*** *18; nb. 1; (2018); Art.No: 52*

Abstract: Background: Breast cancer is one of the most commonly diagnosed invasive cancers among women around the world. Among severalsubtypes, triple negative breast cancer (TNBC) is highly aggressive and chemoresistant. Treatment of TNBC patients has been challengingdue to heterogeneity and devoid of well-defined molecular targets. Thus, identification of novel effective and selective agents against TNBC isessential. Methods: We used epoxyazadiradione to assess the cell viability, mitochondrial potential, ROS level, cell migration, apoptosis andprotein expression in cell culture models of TNBC MDA-MB-231 and ER+ MCF-7 breast cancer cells. The molecular mechanism was examinedin two different type of breast cancer cells in response to epoxyazadiradione. We have also analyzed the effect of epoxyazadiradione onbreast tumor growth using in vivo mice model. Results: In this study, we for the first time investigated that out of 10 major limonoids isolatedfrom Azadirachta indica, epoxyazadiradione exhibits most potent anti-cancer activity in both TNBC and ER+ breast cancer cells. Epoxyazadiradione induces apoptosis and inhibits PI3K/Akt-mediated mitochondrial potential, cell viability, migration and angiogenesis. It also inhibits theexpression of pro-angiogenic and pro-metastatic genes such as Cox2, OPN, VEGF and MMP-9 in these cells. Furthermore, epoxyazadiradioneattenuates PI3K/Akt-mediated AP-1 activation. Our in vivo data revealed that epoxyazadiradione suppresses breast tumor growth and angiogenesisin orthotopic NOD/SCID mice model. Conclusion: Our findings demonstrate that epoxyazadiradione inhibits PI3K/Akt-dependent mitochondrialdepolarisation, induces apoptosis and attenuates cell migration, angiogenesis and breast tumor growth suggesting that this compound may act as a potent therapeutic agent for the management of breast cancer.

Protective role of nimbolide against chemotherapeutic drug hydroxyurea induced genetic and oxidative damage in an animal model
Environmental Toxicology and Pharmacology; ***vol.*** *60; (2018); p. 91 – 99*

Abstract: Nimbolide is known to be an antioxidant found in neem plant. Hydroxyurea is a medication frequently used in sickle-cell disease,different cancers and HIV infection. The present study aimed to evaluate the adverse effect of HU and possible amelioration by nimbolide inWistar rats. To test our hypothesis, we performed genotoxicity tests, biochemical assays, and histopathological studies. We observed that HUcaused higher levels of genotoxicity in the treated animals. The observed genetic and oxidative damage might be due to the presence of reactivespecies as HU increased the level of the malondialdehyde-a biomarker of oxidative damage. Interestingly, co-treatment of animals with HU and nimbolide showed a lower level of damage. We conclude that nimbolide significantly protects the cells from the adverse effect of HU and couldbe considered as a potential adjuvant for the patients under HU therapy.

A supercritical CO2 extract of neem leaf (A. indica) and its bioactive liminoid, nimbolide, suppresses colon cancer in preclinical models by modulating pro-inflammatory pathways
*Molecular Carcinogenesis; **vol.** 57; nb. 9; (2018); p. 1156 – 1165*

Abstract: Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death in men andwomen in the United States. Anti-inflammatory blockade has been proven to be a promising avenue of colorectal cancer prevention. However,NSAIDs while effective in curbing CRC risk are too toxic for long-term use in cancer prevention. The Neem tree (Azadirachta indica) is rich inliminoid terpenoids, collectively known as azadiractoids and has been shown to have anti-inflammatory effects. To explore a role of neem inCRC, human colon cancer cell lines HCT116 and HT29 cells were treated with purified Super Critical Neem Extract (SCNE) or the neemliminoid, nimbolide. SCNE treatment resulted in a dose dependent inhibition of CRC cell proliferation and an increase in apoptosis. Treatmentwith SCNE and nimbolide decreased the expression of transcriptional factors, STAT3 and NF- B which plays a major role in gene regulation ofmultiple cellular processes. Protein expression of COX1, IL-6, and TNF- were decreased on treatment with SCNE in CRC cells. Western blotsand Zymogram assays results revealed anti-invasive effect by decreased expression of MMP2 and MMP9 proteins in CRC cells. Overall, thesedata confirm a potential anti-cancer effect of SCNE, reducing cell proliferation, inflammation, migration, and invasion in human colon cancercells. Confirming these indications, we found that treatment of mice bearing HT29 and HCT116 xenografted tumors exhibited striking inhibitionof colon tumor growth. Clearly we must explore the effect of neem in preclinical animal models for anti-cancer therapy.

Nimbolide epigenetically regulates autophagy and apoptosis in breast cancer
*Toxicology in Vitro; **vol.** 51; (2018); p. 114 – 128*

Abstract: Autophagy is a critical regulator of cellular homeostasis and its dysregulation often results in various disease manifestations, includingcancer. Nimbolide, an active chemical constituent of neem (Azadirachta indica) exhibits potent anticancer effects. Although, nimbolidemediated apoptosis activation in breast cancer cells is well known. Nevertheless, its role in autophagy induction mechanism and epigeneticalteration is not explored previously. Our current study intended to bridge the gaps in the existing research by exploring the potential of nimbolidein inducing autophagy, which could counter regulate the transformations in breast cancer. In our studies, nimbolide significantly inhibitedthe cell proliferation of MDA-MB-231 and MCF-7 cells with IC50 values of 1.97 •} 0.24 and 5.04 •} 0.25 μM, respectively. Nimbolide markedlyarrested the cell cycle progression and cell survival with loss of mitochondrial membrane potential by reducing Bcl-2 concomitantly inducingBax and caspases protein expression with modulation of HDAC-2 and H3K27Ac expression. Consequently, characteristic autophagolysosomeaccumulation was observed by acridine orange, monodansylcadaverine (MDC) and Lysotracker Red staining. Moreover, nimbolide inducedautophagy signaling by increasing Beclin 1 and LC3B along with decreased p62 and mTOR protein expression. Thus, our findings imply thatnimbolide induces autophagy mediated apoptotic cell death in breast cancer with epigenetic modifications.

First report on the pharmacokinetic profile of nimbolide, a novel anticancer agent in oral and intravenous administrated rats by LC/MS method
*Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences; **vol.** 1092; (2018); p. 191 – 198*

Abstract: Nimbolide is a novel, natural compound with promising potential as a drug candidate for anticancer activity. It is isolated from theIndian traditional medicinal plant Azadirachta indica popularly known as neem. The present study was undertaken to explore the oral bioavailabilityand pharmacokinetic

characteristics of nimbolide in rats using the LC/QTOF/MS method. A simple protein precipitation method usingacetonitrile was employed for extracting nimbolide from rat plasma. The chromatographic separation of nimbolide and the internal standard(regorafenib) was attained on an Aquity BEH C18 column (100 × 2.1 mm, 2.7 μm), using ACN and 0.1percent of formic acid in water asmobile phase components in a gradient elution mode at a flow rate of 0.45 mL/min over a short run time of 4 min. A mass detection wascarried out using target ions of [M + H]+ at m/z 467.2074 for nimbolide and m/z 483.0847 for the internal standard. The LC/MS method wasvalidated and all the parameters were found well within the specified limits. The calibration curve was constructed in the range of 1–1000ng/mL. The method shows good accuracy (91.66–97.12percent) and precision (intra 2.21–6.92percent CV and inter-day 2.56–4.62percent CV).This developed LC/MS method was effectively applied to the pharmacokinetic study of nimbolide upon oral and intravenous administration inrats. In concordance with its physicochemical properties and high lipophilicity, we found that it shows poor oral absorption at different doses(10, 30 and 50 mg/kg). As expected, higher plasma levels were observed upon intravenous (10 mg/kg) administration. This method can beextended for evaluation of drug interaction and drug metabolism in rats as well as in humans. Moreover, our rapid and sensitive method maycater the need to accelerate the preclinical formulation development and lead optimization for future drug development of this potent anticanceragent. Further, our oral bioavailability studies demonstrated that nimbolide possesses poor oral absorption, which could be the probable reasonfor the delay in therapeutic translation of this promising agent for clinical use.

Nimbolide induced apoptosis by activating ERK-mediated inhibition of c-IAP1 expression in human hepatocellular carcinoma cells

Environmental Toxicology; ***vol.*** *33; nb. 9; (2018); p. 913 – 922*

Abstract: Nimbolide is one of the major compounds from the leaves and flowers of the neem tree and exhibits antitumor properties on variouscancer cells. However, no report has shown that nimbolide induces apoptosis in vitro and in vivo in human hepatocellular carcinoma cells. Ourresults indicated that it inhibited cell growth in Huh-7 and PLC/PRF/5 cells. We also found that nimbolide induced cell death through the inductionof G2/M phase arrest and mitochondrial dysfunction, accompanied by the increased expression of cleaved caspase-7, caspase-9, caspase-3, caspase-PARP, and Bax and decreased expression of Mcl-1 and Bcl-2. A human apoptosis antibody array analysis demonstrated thatinhibition of the apoptosis family proteins (XIAP, c-IAP1, and c-IAP2) was one of the major targets of nimbolide. Additionally, nimbolidesustained activation of ERK expression. Moreover, pretreatment with U0126 (MEK inhibitor) markedly abolished nimbolide-inhibited cell viability,induced cell apoptosis, ERK phosphorylation, cleaved caspase-9, caspase-3, cleaved-PARP activation, and increased c-IAP1 expression in Huh-7 cells. An in vivo study showed that nimbolide significantly reduced Huh-7 tumor growth and weight in a xenograft mouse model.This study indicated the antitumor potential of nimbolide in human hepatocellular carcinoma cells.

Antiproliferative activity of neem leaf extracts obtained by a sequential pressurized liquid extraction

Pharmaceuticals; ***vol.*** *11; nb. 3; (2018); Art.No: 76*

Abstract: Azadirachta indica A. Juss (neem) extracts have been used in pharmaceutical applications as antitumor agents, due to their terpenesand phenolic compounds. To obtain extracts from neem leaves with potential antiproliferative effect, a sequential process of pressurized liquidextraction was carried out in a fixed bed extractor at 25 C and 100 bar, using hexane (SH), ethyl acetate (SEA), and ethanol (SE) as solvents.Extractions using only ethanol (EE) was also conducted to compare the characteristics of the fractionated extracts. The results obtained byliquid chromatography-electrospray ionization mass spectrometry suggested a higher concentration of terpenes in the SEA extract in comparisonto SH, SE,

and EE extracts. Therefore, antiproliferative activity showed that SEA extracts were the most efficient inhibitor to human tumorcells MCF-7, NCI-H460, HeLa, and HepG2. Hepatocellular cells were more resistant to SH, SEA, SE, and EE compared to breast, lung, hepatocellular,and cervical malignant cells. Neem fractioned extracts obtained in the present study seem to be more selective for malignant cells

A sensitive liquid chromatography-tandem mass spectrometry method for the determination of nimbolide in mouse serum: Application to a preclinical pharmacokinetics study
Pharmaceutics; ***vol.*** *10; nb. 3; (2018); Art.No: 123*

Abstract: A sensitive and robust liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed and validated for thedetermination of nimbolide in mouse serum. Exemestane was used as the internal standard (IS). Here, we employed acetonitrile-based proteinprecipitation (PPT) for serum sample preparation, and performed chromatographic separation using an ODS Hypersil C18 column (100 mm ×2.1 mm, 5 μm) with gradient elution (0.1percent formic acid in water vs 100percent acetonitrile). The run time was 6 min. Instrumental analysiswas performed by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in the multiple-reaction monitoring (MRM) under positivemode. A good linear calibration was achieved in the 5–1000 ng/mL range. The intra-and inter-day precisions for nimbolide were ≤12.6percentand ≤13.9percent respectively. Intra-day accuracy ranged from 96.9–109.3percent, while inter-day accuracy ranged from 94.3–110.2percent.The matrix effect of nimbolide, detected but consistent at low and high concentrations, do not affect linearity of standard curve. In conclusion,we have developed and validated a sensitive analytical method for determination of a novel natural compound nimbolide in mouse serum,and it has been successfully applied to our preclinical study in investigating the pharmacokinetic properties of nimbolide, which could greatlyfacilitate the preclinical development of the promising lead compound for anticancer therapy.

Inhibition of cell survival and proliferation by nimbolide in human androgen-independent prostate cancer (PC-3) cells: involvement of the PI3K/Akt pathway
Molecular and Cellular Biochemistry; ***vol.*** *427; nb. 1-2; (2017); p. 69 – 79*

Abstract: Prostate cancer is most common malignancy among men in the world. PI3K-Akt signaling appears to be critical to prostate cancercell proliferation and survival. Our earlier study reveals that nimbolide (2 μM) prevents cell survival via IGF signaling pathway throughPI3K/Akt and induces apoptosis in PC-3 cell line. Akt mediates the phosphorylation and activation of mTOR that plays a critical role in the regulation of protein translation and synthesis, angiogenesis, and cell cycle progression. The present study was aimed to investigate the effectof nimbolide on tPI3K, tAkt, pAkt, tmTOR, GSK3 , pGSK3 , PCNA, c-Myc, Cyclin D1, and Survivin protein levels by western blot analysis.Apoptosis was visualized by Ao/EtBr dual staining (20×), and protein expression of PCNA by immunocytochemistry was performed. Moleculardocking was performed to understand the possible interaction between nimbolide and Akt, PCNA, and Cyclin D1. Nimbolide altered thePI3K-Akt-mediated cell survival and proliferative molecules. Thus, nimbolide exerted anticancer effects in vitro by representing the PI3K-AktmTORpathway in PC-3 cells. Thereby, it acts as a potent anticancer drug for prostate cancer.

Gedunin inhibits pancreatic cancer by altering sonic hedgehog signaling pathway
Oncotarget; ***vol.*** *8; nb. 7; (2017); p. 10891 – 10904*

Abstract: INTRODUCTION: The lack of efficient treatment options for pancreatic cancer highlights the critical need for the development ofnovel and effective chemotherapeutic agents. The medicinal properties found in plants have been used to treat many different illnesses includingcancers. This study focuses on the anticancer effects of gedunin, a natural compound isolated from Azadirachta indica. METHODS: Antiproliferativeeffect of gedunin on pancreatic cancer cells was assessed using MTS assay.

We used matrigel invasion assay, scratch assay, andsoft agar colony formation assay to measure the anti-metastatic potential of gedunin. Immunoblotting was performed to analyze the effect ofgedunin on the expression of key proteins involved in pancreatic cancer growth and metastasis. Gedunin induced apoptosis was measured usingflow cytometric analysis. To further validate, xenograft studies with HPAC cells were performed. RESULTS: Gedunin treatment is highly effectivein inducing death of pancreatic cancer cells via intrinsic and extrinsic mediated apoptosis. Our data further indicates that gedunin inhibitedmetastasis of pancreatic cancer cells by decreasing their EMT, invasive, migratory and colony formation capabilities. Gedunin treatmentalso inhibited sonic hedgehog signaling pathways. Further, experiments with recombinant sonic hedgehog protein and Gli inhibitor (Gant-61)demonstrated that gedunin induces its anti-metastatic effect through inhibition of sonic hedgehog signaling. The anti- cancer effect of geduninwas further validated using xenograft mouse model. CONCLUSION: Overall, our data suggests that gedunin could serve as a potent anticanceragent against pancreatic cancers.

Nimbolide upregulates RECK by targeting miR-21 and HIF-1 in cell lines and in a hamster oral carcinogenesis model
Scientific Reports; ***vol.*** *7; nb. 1; (2017); Art.No: 2045*

Abstract: Reversion-inducing cysteine-rich protein with Kazal motifs (RECK), a potent inhibitor of matrix metalloproteinases (MMPs) is acommon negative target of oncogenic signals and a potential therapeutic target for novel drug development. Here, we show that sequentialRECKlessness stimulates angiogenesis and Notch signalling in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch(HBP) carcinogenesis model, a paradigm for oral oncogenesis and chemointervention. We also report the chemotherapeutic effect of nimbolide,a limonoid from the neem tree (Azadirachta indica) based on the upregulation of RECK as well as modulation of the expression of key moleculesinvolved in invasion and angiogenesis. We demonstrate that nimbolide upregulates RECK by targeting miR-21, and HIF-1 resulting inreduced MMP activity and blockade of VEGF and Notch signalling. Nimbolide reduced microvascular density, confirming its anti-angiogenicpotential. Molecular docking analysis revealed interaction of nimbolide with HIF-1 . Additionally, we demonstrate that nimbolide upregulatesRECK expression via downregulation of HIF-1 and miR-21 by overexpression and knockdown experiments in SCC4 and EAhy926 cell lines.Taken together, these findings provide compelling evidence that targeting RECK, a keystone protein that regulates mediators of invasion andangiogenesis with phytochemicals such as nimbolide may be a robust therapeutic approach to prevent oral cancer progression.

Nimbolide suppresses non-small cell lung cancer cell invasion and migration via manipulation of DUSP4 expression and ERK1/2 signaling
Biomedicine and Pharmacotherapy; ***vol.*** *92; (2017); p. 340 – 346*

Abstract: Nimbolide plays an important role in treating human diseases. In these years, the anticancer property of nimbolide has been paidmore and more attention. However, the role of nimbolide in non-small cell lung cancer (NSCLC) remains unclear. In this study, we found thatnimbolide treatment suppressed the invasion and migration of NSCLC cells, in a dose-dependent manner. Moreover, nimbolide treatment dosedependentlyinhibited ERK1/2 activation, decreased Snail and MMP-3 expression, and increased E-cadherin expression. Further, we found thatnimbolide treatment upregulated DUSP4 expression. DUSP4 knockdown attenuated nimbolide-mediated inhibition of cell invasion, migrationand ERK1/2 activation. We also found that DUSP4 knockdown suppressed the effect of nimbolide on MMP-3, Snail and E-cadherin expression.Taken together, our study demonstrates that nimbolide treatment can upregulate the expression of DUSP4, thus inhibiting ERK1/2 activation.Inhibition of ERK1/2 pathway by nimbolide decreases MMP-3 and Snail expression, and increases E-cadherin expression, which finallyinhibits NSCLC cell invasion and migration. Therefore, nimbolide may act as a novel drug to inhibit NSCLC invasion and metastasis throughmanipulation of ERK1/2 signaling and DUSP4 expression.

Bijauliya, Rohit Kumar; Alok, Shashi; Singh, Man; Mishra, Shanti Bhushan

A comprehensive review on cancer and anticancer herbal drugs
International Journal of Pharmaceutical Sciences and Research; ***vol.*** *8; nb. 7; (2017); p. 2740 – 2761*

Abstract: Cancer is a major public health burden in both developed and developing countries. It is an abnormal growth of cells in body thatcan lead to death and globally the numbers of cancer patients are increasing day by day. There are several medicines available in the market totreat the various types of cancer but no drug is found to be fully effective and safe. Herbal medicines have a vital role in the prevention andtreatment of cancer. With advanced knowledge of molecular science and refinement in isolation and structure elucidation techniques, variousanticancer herbs has been identified, which execute their therapeutic effect by inhibiting cancer-activating enzymes and hormones, stimulatingDNA repair mechanism, promoting production of protective enzymes, inducing antioxidant action and enhancing immunity of the body. Plantshave been used for treating diseases since time immemorial. More than 50percent of modern drugs in clinical use are of natural products. In thepresent review, an attempt has been made to study the plants that have been used in the treatment of cancer.

Nimbolide induces apoptosis in human nasopharyngeal cancer cells
Environmental Toxicology; ***vol.*** *32; nb. 8; (2017); p. 2085 – 2092*

Abstract: Nasopharyngeal carcinoma (NPC), a tumor arising from epithelial cells that cover the surface and line the nasopharynx, is a raremalignancy worldwide but is prevalent in certain geographical areas, such as Southern Asia (Taiwan, Hong Kong, Singapore, Malaysia, andSouthern China) and North Africa. Despite advances in diagnostic techniques and improvements in treatment modalities, the prognosis of NPCremains poor. Therefore, an effective chemotherapy regimen that enhances tumor sensitivity to chemotherapeutics is urgently required. Nimbolide,derived from Azadirachta indica, has a wide range of beneficial effects, including anti-inflammatory and anticancer properties. Thepresent study evaluated the antitumor activity of nimbolide in NPC cells and its underlying mechanisms. Our results revealed that the treatmentof HONE-1 cells with nimbolide potently inhibited cell viability. Moreover, nimbolide led to cell cycle arrest, which subsequently activatedcaspase-3, −8, and −9 and poly (ADP-ribose) polymerase to induce cell apoptosis. Moreover, nimbolide induced Bik, Bax, and t-Bid expressionin HONE-1 cells. The results indicated that nimbolide induces apoptosis through the modulation of extracellular signal-regulated kinases 1and 2 (ERK1/2) pathways. Nimbolide induces apoptosis in human NPC cells and is a potential chemopreventive agent against NPC proliferationSiva, Bandi; Devi, Amujuri; Venkanna, Arramsetti; Poornima, Borra; Sukumar, Genji; Reddy, Solipeta Divya; Vijaya, Movva; Ummanni, Ramesh; Babu, Katragadda Suresh

"Click" reaction based synthesis of nimbolide derivatives and study of their insect antifeedant activity against Spodoptera litura Larvae
Fitoterapia; ***vol.*** *123; (2017); p. 1 – 8*

Abstract: A series of Nimbolide-triazole conjugates were synthesized through copper(I)- catalyzed azide–alkyne "click" chemistry approachand these derivatives (2–4, 2a–2l) were characterized using modern spectroscopic techniques. Antifeedant activities of these derivatives werestudied on Tobacco Caterpillar, Spodoptera litura (F.) using no-choice leaf disk bioassay. Interestingly, the synthesized derivatives were moreeffective in reducing feedancy by insect species when compared to the parent nimbolide. Among the tested compounds, 2a, 2c, and 2d showedpotent antifeedancy with ED50 values of 0.49, 0.95 and 0.97 mg/cm2 against S. litura. Several of the analogs were also toxic or caused developmentalabnormalities following leaf disc assay.

Epithelial mesenchymal transition in cancer progression: Preventive phytochemicals

Recent Patents on Anti-Cancer Drug Discovery; ***vol.*** *12; nb. 3; (2017); p. 234 – 246*

Abstract: Background: Epithelial-Mesenchymal Transition (EMT) is the conversion of epithelial cells into mesenchymal phenotype generallyobserved during embryogenesis and wound healing as well as in malignant transformation. Several signaling pathways and transcription factorsassociated with EMT have been explored. Dietary phytochemicals that are multi-targeted agents which interfere with these pathways, assumepreventive potential against pathologic EMT. Objective: The present review aims to provide a detailed description of the nature and characteristicsof EMT in physiological and pathophysiological conditions and the scope of phytochemicals in its prevention. Method: Details regardingthe initiation, progression as well as prevention of pathologic EMT and metastasis and recent patents on preventive phytochemicals were obtainedfrom PubMed literatures and patent databases. Results: The phenotypic changes during EMT are regulated by transcription factors likeSnail, Slug, Twist and Zeb, which are activated through diverse signaling pathways of TGF- , NF- B, Wnt and Notch. Scientific documentationstill date have identified numerous phytocompounds that are potent enough to interfere with these signaling pathways, which in turn preventpathological implications of EMT. Present review also discusses 28 recent patents on those phytocompounds. Conclusion: EMT is a significantpharmacological target for developing preventive agents to combat pathological conditions like malignancy. Many of the phytochemicals citedin this review are being enrolled for different phases of clinical trials for their efficacy. In spite of the major limitations regarding bioavailability,sensitivity and tolerance of these compounds, their synthetic analogs, formulations and efficient drug delivery systems are also being attemptedwhich will hopefully generate productive and promising results in near future.

Nimbolide attenuate the lipid accumulation, oxidative stress and antioxidant in primary hepatocytes

Molecular Biology Reports; ***vol.*** *44; nb. 6; (2017); p. 463 – 474*

Abstract: Nimbolide is a bioactive compound found in Azadirachta indica. This work was devised to investigate the potential effects of nimbolideon intracellular lipid deposition and its associated redox modulation in primary hepatocytes (Heps). Lipid accumulation was induced inHeps by supplementing 1 mM oleic acid for 24 h which was marked by significant accumulation of lipids. The results demonstrated that nimbolidecan decrease intracellular cholesterol, free fatty acids and triglycerides. Nimbolide may also improve hepatocytes function through itsantioxidant effects by inhibiting oxidative DNA damage and lipid peroxidation by curtailing the reactive oxygen species levels. Further it alsorestore the mitochondrial potential, improving the endogenous antioxidant levels such as GSH and antioxidant enzyme activities. Nimbolideincreased ($P < 0.05$) liver X receptor- (LXR), peroxisome proliferator-activated receptor- (PPAR) and sterol regulatory element-binding protein-1c (SREBP1c) gene expression in Heps. The biological significance of nimbolide may involve hypolipidemic effect, lipid peroxidationinhibition, DNA damage inhibition, ROS inhibition, restoring mitochondrial function, increases in GSH and SOD and CAT activities, and directregulation of LXR , PPAR and SREBP1c gene expression. Nimbolide may be used as effective lipid lowering compound and lipid deposition-induced Heps changes.

Nimbolide inhibits pancreatic cancer growth and metastasis through ROS-mediated apoptosis and inhibition of epithelial-to-mesenchymal transition

Scientific Reports; ***vol.*** *6; (2016); Art.No: 19819*

Abstract: The mortality and morbidity rates of pancreatic cancer are high because of its extremely invasive and metastatic nature. Its lack ofsymptoms, late diagnosis and chemo-resistance and the ineffective treatment modalities warrant the development of new chemo-therapeuticagents for pancreatic cancer. Agents from medicinal plants have demonstrated therapeutic benefits in various human cancers. Nimbolide, anactive molecule isolated from Azadirachta indica, has been reported to exhibit several

medicinal properties. This study assessed the anticancerproperties of nimbolide against pancreatic cancer. Our data reveal that nimbolide induces excessive generation of reactive oxygen species(ROS), thereby regulating both apoptosis and autophagy in pancreatic cancer cells. Experiments with the autophagy inhibitors 3-methyladenineand chloroquine diphosphate salt and the apoptosis inhibitor z-VAD-fmk demonstrated that nimbolide-mediated ROS generation inhibited proliferation(through reduced PI3K/AKT/mTOR and ERK signaling) and metastasis (through decreased EMT, invasion, migration and colonyforming abilities) via mitochondrial-mediated apoptotic cell death but not via autophagy. In vivo experiments also demonstrated that nimbolidewas effective in inhibiting pancreatic cancer growth and metastasis. Overall, our data suggest that nimbolide can serve as a potential chemotherapeuticagent for pancreatic cancer.

Nimbolide, a neem limonoid inhibits Phosphatidyl Inositol-3 Kinase to activate Glycogen Synthase Kinase-3 in a hamster model of oral oncogenesis
Scientific Reports; ***vol.*** *6; (2016); Art.No: 22192*

Abstract: Glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase is frequently inactivated by the oncogenic signalling kinasesPI3K/Akt and MAPK/ERK in diverse malignancies. The present study was designed to investigate GSK-3 signalling circuits in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis model and the therapeutic potential of the neem limonoidnimbolide. Inactivation of GSK-3 by phosphorylation at serine 9 and activation of PI3K/Akt, MAPK/ERK and -catenin was associated withincreased cell proliferation and apoptosis evasion during stepwise evolution of HBP carcinomas. Administration of nimbolide inhibitedPI3K/Akt signalling with consequent activation of GSK-3 thereby inducing trafficking of -catenin away from the nucleus and enhancing theexpression of miR-126 and let-7. Molecular docking studies confirmed interaction of nimbolide with PI3K, Akt, ERK and GSK-3 . Furthermore,nimbolide attenuated cell proliferation and induced apoptosis as evidenced by increased p-cyclin D1Thr286 and pro-apoptotic proteins.The present study has unravelled aberrant phosphorylation as a key determinant for oncogenic signalling and acquisition of cancer hallmarks inthe HBP model. The study has also provided mechanistic insights into the chemotherapeutic potential of nimbolide that may be a useful additionto the armamentarium of natural compounds targeting PI3K for oral cancer treatment.

Nimbolide-Induced Oxidative Stress Abrogates STAT3 Signaling Cascade and Inhibits Tumor Growth in Transgenic Adenocarcinoma of Mouse Prostate Model
Antioxidants and Redox Signaling; ***vol.*** *24; nb. 11; (2016); p. 575 – 589*

Abstract: Aims: Prostate cancer (PCa) is one of the most commonly diagnosed cancers worldwide. Currently available therapies for metastaticPCa are only marginally effective, hence novel treatment modalities are urgently required. Considerable evidence(s) suggest that deregulatedactivation of oncogenic transcription factor, signal transducer and activator of transcription 3 (STAT3) plays a pivotal role in the developmentand progression of PCa. Thus, agents that can abrogate STAT3 activation could form the basis of novel therapy for PCa patients. In the presentstudy, we analyzed whether the potential anticancer effects of nimbolide (NL), a limonoid triterpene derived from Azadirachta indica, againstPCa cell lines and transgenic adenocarcinoma of mouse prostate (TRAMP) model are mediated through the negative regulation of STAT3 pathway.Results: Data from the in vitro studies indicated that NL could significantly inhibit cell viability, induce apoptosis, and suppress cellularinvasion and migration. Interestingly, NL also abrogated STAT3 activation and this effect was found to be mediated via an increased productionof reactive oxygen species (ROS) due to GSH/GSSG imbalance. Oral administration of NL significantly suppressed the tumor growth andmetastasis in TRAMP mouse model without exhibiting any significant adverse effects. Innovation: The present study demonstrates the criticalrole of GSH/GSSG imbalance-mediated ROS production contributing to the STAT3 inhibitory and tumor-suppressive effect of NL in PCa.Conclusion: Overall, our findings indicate that NL exhibits significant anticancer effects in PCa that may be primarily mediated through theROS-regulated inhibition of STAT3 signaling cascade. Antioxid. Redox Signal. 24, 575-589.

Combination of nimbolide and TNF- -increases human colon adenocarcinoma cell death through JNK-mediated DR5 upregulation
Asian Pacific Journal of Cancer Prevention; ***vol.*** *17; nb. 5; (2016); p. 2637 – 2641*

Abstract: Tumor necrosis factor (TNF-), an inflammatory cytokine that plays an important role in the control of cell proliferation, differentiation,and apoptosis, has previously been used in anti-cancer therapy. However, the therapeutic applications of TNF- are largely limited due to itsgeneral toxicity and anti-apoptotic influence. To overcome this problem, the present study focused on the effect of active constituents isolatedfrom a medicinal plant on TNF- -induced apoptosis in human colon adenocarcinoma (HT-29) cells. Nimbolide from Azadirachta indica wasevaluated for cytotoxicity by methyl tetrazolium 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay and phase contrastmicroscopy. Effects on apoptotic signaling proteins were investigated using Western blot analysis. Nimbolide showed cytotoxicity againstHT-29 cells that was significantly different from the control group ($p < 0.01$), a concentration of 10 μM significantly inducing cell death ($p < 0.01$). In combination with TNF- , nimbolide significantly enhanced-induced cell death. In apoptotic pathway, nimbolide activated c-Jun N-terminalkinase (JNK) phosphorylation, BH3 interacting-domain death agonist (Bid) and up-regulated the death receptor 5 (DR5) level. In thecombination group, nimbolide markedly sensitized TNF- -induced JNK, Bid, caspase-3 activation and the up-regulation of DR5. Our findingsoverall indicate that nimbolide may enhance TNF- -mediated cellular proliferation inhibition through increasing cell apoptosis of HT-29 cells byup-reglation of DR5 expression via the JNK pathway

Anticancer properties of nimbolide and pharmacokinetic considerations to accelerate its development
Oncotarget; ***vol.*** *7; nb. 28; (2016); p. 44790 – 44802*

Abstract: Nimbolide is one of the main components in the leaf extract of Azadirachta indica (A. indica). Accumulating evidence from variousin vitro and in vivo studies indicates that nimbolide possesses potent anticancer activity against several types of cancer and also shows potentialchemopreventive activity in animal models. The main mechanisms of action of nimbolide include anti-proliferation, induction of apoptosis,inhibition of metastasis and angiogenesis, and modulation of carcinogen-metabolizing enzymes. Although multiple pharmacodynamic (PD)studies have been carried out, nimbolide is still at the infant stage in the drug development pipeline due to the lack of systematic pharmacokinetic(PK) studies and long-term toxicological studies. Preclinical PK and toxicological studies are vital in determining the dosage range tosupport the safety of nimbolide for first-in-human clinical trials. In this review, we will provide a comprehensive summary for the current statusof nimbolide as an anticancer and chemopreventive lead compound, and highlight the importance of systematic preclinical PK and toxicologicalstudies in accelerating the process of application of nimbolide as a therapeutic agent against various malignancies.

Correction: Nimbolide sensitizes human colon cancer cells to TRAIL through reactive oxygen species- and ERK-dependent up-regulation of death receptors, p53, and Bax
Journal of Biological Chemistry; ***vol.*** *291; nb. 32; (2016); p. 16925 – 16925*

Abstract: This article has been retracted by the publisher. An investigation at MD Anderson determined that the image of medium control andthe image of DR4+DR5 siRNA treated with TRAIL in Fig. 3B were reused.

Nimbolide Inhibits Nuclear Factor- B Pathway in Intestinal Epithelial Cells and Macrophages and Alleviates Experimental Colitis in Mice
Phytotherapy Research; ***vol.*** *30; nb. 10; (2016); p. 1605 – 1614*

Abstract: Nimbolide is a limonoid extracted from neem tree (Azadirachta indica) that has antiinflammatory properties. The effect of nimbolideon the nuclear factor-kappa B (NF- B) pathway in intestinal epithelial cells (IECs), macrophages and in murine colitis models was investigated.The IEC COLO 205, the murine macrophage cell line RAW 264.7, and peritoneal macrophages from interleukin-10-deficient (IL-10−/−) micewere preconditioned with nimbolide and then stimulated with tumor necrosis factor- (TNF-) or lipopolysaccharide. Dextran sulfate sodiuminducedacute colitis model and chronic colitis model in IL-10−/− mice were used for in vivo experiments. Nimbolide significantly suppressedthe expression of inflammatory cytokines (IL-6, IL-8, IL-12, and TNF-) and inhibited the phosphorylation of I B and the DNA-binding affinityof NF- B in IECs and macrophages. Nimbolide ameliorated weight loss, colon shortening, disease activity index score, and histologic scores indextran sulfate sodium colitis. It also improved histopathologic scores in the chronic colitis of IL-10−/− mice. Staining for phosphorylated I Bwas significantly decreased in the colon tissue after treatment with nimbolide in both models. Nimbolide inhibits NF- B signaling in IECs andmacrophages and ameliorates experimental colitis in mice. These results suggest nimbolide could be a potentially new treatment for inflammatorybowel disease.

Intracellular,biofilm-inhibitory and membranedamaging activities of nimbolide isolated from Azadirachta indica A. Juss (Meliaceae) against meticillin-resistant Staphylococcus aureus
*Journal of Medical Microbiology; **vol.** 65; nb. 10; (2016); p. 1205 – 1214*

Abstract: Staphylococcus aureus is a leading aetiologic agent of nosocomial- and community-acquired infectious diseases worldwide. Thepublic health concern regarding staphylococcal infections is inflated by the increasing occurrence of multidrug-resistant strains,e.g. multidrugandmeticillinresistant S. aureus (MDR MRSA). This study was designed to evaluate the intracellular killing,membrane-damaging and biofilminhibitoryactivities of nimbolide isolated from Azadirachta indica against MDR MRSA. In vitro antibacterial activity of nimbolide was determinedby performing MIC,minimal bactericidal concentration (MBC) and time-kill kinetic studies. Bacterial membrane-damaging activity wasdetermined by membrane perturbation and scanning electron microscopy (SEM) examination. Biofilm-inhibitory activities were determined bySEM. Cellular drug accumulation and assessments of intracellular activities were performed using Vero cell culture. SEM revealed that nimbolidecaused significant membrane damage and lysis of the S. aureus cells. The biofilm structure was disrupted,and the biofilm formation wasgreatly reduced in the presence of nimbolide as examined by SEM. The level of accumulation of nimbolide in Vero cells incubated for 24 h isrelatively higher than that of ciprofloxacin and nalidixic acid (Cc/Ce for nimbolide>ciprofloxacin and nalidixic acid). The viable number ofintracellular S. aureus was decreased [reduction of ~2 log10 c.f.u. (mg Vero cell protein)-1] in a time-dependent manner in the presence of nimbolide(4× MBC) that was comparable to that of tetracycline and nalidixic acid. The significant intracellular,biofilm-inhibitory and bacterialmembrane-damaging activities of nimbolide demonstrated here suggested that it has potential as an effective antibacterial agent for the treatmentof severe infections caused by MDR MRSA.

Nimbolide inhibits androgen independent prostate cancer cells survival and proliferation by modulating multiple pro-survival signaling pathways
*Biomedicine and Pharmacotherapy; **vol.** 84; (2016); p. 1623 – 1634*

Abstract: Background Prostate cancer is the most prominent cancer in men, experiencing a relapse in disease often express high serum TNFlevels.It has been correlated with increased cell survival and proliferation of prostate cancer cells. Previous studies reported that nimbolide, aterpenoid derived from the leaves and flowers of neem tree inhibits cancer growth through selective modulation of cell signaling pathwayslinked to inflammation, survival, proliferation, angiogenesis and metastasis. Methods The present study aimed to examine the effect of nimbolideat 1 and 2 μM concentrations on TNF- /TNFR1

mediated signaling molecules involved in cell survival and proliferation in PC-3 cell linevia NF- B and MAPK pathways by real time PCR and western blot. Protein and compound interaction were performed by Molecular dockinganalysis. Results Our results indicate that nimbolide treatment suppressed expression of TNF- , SODD, Grb2, SOS mRNA and modulated TNF-/TNFR1 regulated NF- B and MAPK signaling molecules in PC-3 cells. Additional molecular dynamics simulation studies confirmed the stabilityof nimbolide and signaling molecules binding interactions. Binding pose analysis revealed the significance of hydrogen bond interactionsfor effective stabilization of virtual ligand protein complexes. Conclusion Nimbolide inhibited prostate cancer cell survival and proliferation viaNF- B and MAPK pathways.

NEEM COMPOSITIONS USED FOR THE TREATMENT OF CANCER
Patent: *WO2015/35199; (2015); (A1)*
Abstract: The present invention includes compositions and methods of treating one or more symptom of cancer by administering a therapeuticallyeffective dose of a pharmaceutical formulation, selected from Nimbolide, Nimbandiol, 2', 3 ' dihydro Nimbolide, 28 dihydro Nimbolide,or a combination thereof to the patient to ameliorate one or more symptoms of the cancer; and monitoring the one or more symptom of thecancer.

Selective induction of apoptosis by Azadarichta indica leaf extract by targeting oxidative vulnerabilities in human cancer cells
Journal of Pharmacy and Pharmaceutical Sciences; ***vol.*** *18; nb. 4; (2015); p. 729 – 746*

Abstract: Purpose: Natural products have been a great source of medications used in conventional medicines for the treatment of various diseases;more importantly, they have played a significant role in the development of anti-cancer drugs for a number of decades. The benefits toemploying whole extracts of natural health products, rather than a single ingredient, for cancer treatment remains unexplored. Our researchgroup has previously demonstrated the potential anti-cancer benefits of several natural health products (NHPs), prompting further studies intoother NHPs, such as Neem (Azadarichta indica), a tree native to India and has been used in Ayurvedic medicine for over 4000 years. Theobjective of this study is to determine the possible anti-cancer potential of aqueous and ethanolic Neem leaf extracts (NLEs) and to identify thespecific mode(s) of action. Methods: Cells were treated with NLE and cell viability was then assessed using a water-soluble tetrazolium salt.Cell death was confirmed using the fluorescent dye propidium iodide and apoptosis was identified using the Annexin-V binding assay. Mitochondrialmembrane permeabilization was visualized using JC-1 staining and the production of whole cell and mitochondrial ROS was measuredwith 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) and Amplex Red, respectively. In vivo efficacy of aqueous NLE was assessedin human tumour xenografts in CD-1 nu/nu immunocompromised mice. Results: Results indicate that both ethanolic and aqueous extracts ofNeem leaf were effective in inducing apoptosis in leukemia and colon cancer cells, following destabilization of the mitochondrial membrane.Furthermore, an increase in the production of reactive oxygen species (ROS) was observed in cancer cells treated with NLEs, indicating thatoxidative stress may play a role in the mechanism of cell death. Additionally, in vivo results showed that aqueous NLE (delivered orally) waswell tolerated and inhibited tumour growth of human xenografts in mice. Conclusions: These findings suggest the potential of NLEs as saferand effective alternatives to conventional chemotherapy

Cytotoxic and melanogenesis-inhibitory activities of limonoids from the leaves of Azadirachta indica (Neem)
Chemistry and Biodiversity; ***vol.*** *11; nb. 3; (2014); p. 451 – 468*

Abstract: Seventeen limonoids (tetranortriterpenoids), 1-17, including three new compounds, i.e., 17-defurano-17-(2,5-dihydro-2-oxofuran-3-yl)-28-deoxonimbolide (14), 17-defurano-17-(2 -2,5-dihydro-2-hydroxy-5-oxofuran-3-yl)-28- deoxonimbolide (15), and 17-defurano-17-(5 -2,5-dihydro-5-hydroxy-2-oxofuran- 3-yl)-2 ,3 -dehydrosalannol (17), were isolated from an EtOH extract of the leaf of neem (Azadirachta indica).The structures of the new compounds were elucidated on the basis of extensive spectroscopic analyses and comparison with literature. Uponevaluation of the cytotoxic activities of these compounds against leukemia (HL60), lung (A549), stomach (AZ521), and breast (SK-BR-3) cancercell lines, seven compounds, i.e., 1-3, 12, 13, 15, and 16, exhibited potent cytotoxicities with IC50 values in the range of 0.1-9.9 μM againstone or more cell lines. Among these compounds, cytotoxicity of nimonol (1; IC50 2.8 μM) against HL60 cells was demonstrated to be mainlydue to the induction of apoptosis by flow cytometry. Western blot analysis suggested that compound 1 induced apoptosis via both the mitochondrialand death receptor-mediated pathways in HL60 cells. In addition, when compounds 1-17 were evaluated for their inhibitory activitiesagainst melanogenesis in B16 melanoma cells, induced with -melanocyte-stimulating hormone (-MSH), seven compounds, 1, 2, 4-6, 15, and16, exhibited inhibitory activities with 31-94percent reduction of melanin content at 10 μM concentration with no or low toxicity to the cells(82-112percent of cell viability at 10 μM). All 17 compounds were further evaluated for their inhibitory effects against the Epstein-Barr virusearly antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13- acetate (TPA) in Raji cells. Copyright

Nimbolide, a neem limonoid abrogates canonical NF- B and Wnt signaling to induce caspase-dependent apoptosis in human hepatocarcinoma (HepG2) cells
*European Journal of Pharmacology; **vol.** 681; nb. 1-3; (2012); p. 6 – 14*

Abstract: Nuclear factor kappa B (NF- B), an oncogenic signaling factor plays a critical role in the development and progression of variouscancers. The objective of this study was to investigate the effect of nimbolide, a neem derived tetranortriterpenoid on NF- B signaling and itsdownstream events - Wnt/ -catenin activation and apoptosis evasion in human hepatocarcinoma (HepG2) cells by evaluating NF- B family members(NF- B-p50, p65, I B- , p-I B- , and IKK), members of Wnt signaling (GSK-3 and -catenin), and intrinsic apoptosis (Bcl-2, Bax, cytochromec, Smac/DIABLO, caspase-3, and caspase-9). Our results demonstrate that nimbolide concurrently abrogates canonical NF- B and Wnt signalingand induces intrinsic apoptosis in HepG2 cells. These data suggest that phytochemicals such as nimbolide that can target multiple stepsalong the NF- B signaling circuit are promising candidates for future phytochemical-based mechanistic pathway targeted anticancer regimens

Cytotoxic triterpenoids from Azadirachta indica
*Planta Medica; **vol.** 77; nb. 16; (2011); p. 1844 – 1847*

Abstract: Two new tirucallane triterpenoids, 24,25-epoxy-3-hydroxy-20-oxo-7- tirucallene (1) and 22,23;24,25-diepoxy-3-hydroxy-7-tirucallene(2), and a new tetranortriterpenoid, 4-hydroperoxy-6-O-acetylnimbandiol (3), along with eight known compounds, were isolated from thebranches and leaves of Azadirachta indica. Their structures were elucidated through spectroscopic and chemical methods. The cytotoxic assayshowed that the abundant constituent nimbolide (8) had obvious cytotoxic activities against HL-60, SMMC7721, A549, MCF-7, and SW-480cell lines, with IC50 values of 0.8 • }0.1, 2.2 • }0.2, 1.9 • }1.3, 4.5 • }1.1, and 2.3 • }0.1μM, respectively.

Synthesis and biological activity of amide derivatives of nimbolide
*Bioorganic and Medicinal Chemistry Letters; **vol.** 16; nb. 16; (2006); p. 4391 – 4394*

Abstract: Nimbolide (1), a limonoid isolated from Azadirachta indica, is the chief cytotoxic principle in Neem leaves extract. Using nimbolideas a lead compound for anti-cancer analogue design, a series of

nimbolide derivatives have been synthesized and evaluated for in vitro cytotoxicactivity against a panel of human cancer cell lines. Out of 10 compounds screened 2g, 2h and 2i showed potent activity.

The isomeric compounds nimbolide and isonimbolide
Acta Crystallographica Section C: Crystal Structure Communications; ***vol.*** *61; nb. 2; (2005); p. o70-o72*

Abstract: Nimbolide [systematic name: (4 ,5 ,6 ,7 ,15 , 17)-7,15:-21,23-diepoxy-6-hydroxy-4,8-dimethyl-1-oxo-18,24-dinor-11, -12-secochola-2,13,20,22-tetraene-4,11-dicarboxylic acid -lactone methyl ester], C27H30O7, was isolated from the leaves of Azadirachta indica, and its isomer,isonimbolide [systematic name: (4 ,5 ,6 ,7 ,15)-7,15:21,23-diepoxy-6-hydroxy-4, 8-dimethyl-1-oxo-18,24-dinor-11,12-secochola-2,16,20,22-tetraene-4, 11-dicarboxylic acid -lactone methyl ester], was prepared from a novel rearrangement reaction of nimbolide, using boron trifluorideetherate and tetrabutylammonium bromide. The reaction conditions are probably responsible for the ether cleavage, double-bond rearrangementand reformation of the ether linkage. As a result, there are conformational changes in two cyclopentane rings and the side-chain-CH2COOMegroup. In isonimbolide, an R 44(24) hydrogen-bond motif is observed.

Isolation of a new tetranortriterpenoid from the uncrushed green leaves of Azadirachta indica
Phytochemistry; ***vol.*** *52; nb. 6; (1999); p. 1117 – 1119*

Abstract: 14,15-Epoxynimonol, a new tetranortriterpenoid, was obtained from the fresh green whole leaves of Azadirachta indica and its structurehas been assigned on the basis of spectral data. The 14,15-epoxide was assigned - configuration by comparison with cedrelone.

Nimbolide is the prinicipal cytotoxic component of neem-seed insecticide preparations
Pesticide Science; ***vol.*** *48; nb. 2; (1996); p. 135 – 140*

Abstract: Several neem-seed extracts, some used for preparing commercial azadirachtin-containing nsecticides, are cytotoxic to N1E-115murine neuroblastoma cells with IC50 values of 20-200 µg extract ml-1 culture medium. Bioassay-directed fractionation by reversed-phaseHPLC shows that the toxicity to N1E-115 cells is associated primarily with a single minor component identified by isolation and NMR and MSas nimbolide with an IC50 of 1.5 µg ml-1 (3.2 µM). The difference in quantity of nimbolide in seven neem extract sources generally correlateswith their overall cytotoxicity. Three other limonoids (epoxyazadiradione, salannin and possibly deacetylsalannin) but not azadirachtin, nimbinand deacetylnimbin contribute in small part to the cytotoxicity. Reconstituted neem extract with only nimbolide removed is less cytotoxic thanthe original extract. It therefore appears that nimbolide is the principal cytotoxic component of the neem extracts examined and that such minorconstituents may warrant consideration in safety evaluations.

Cytotoxicity of nimbolide, epoxyazadiradione and other limonoids from neem insecticide
Life Sciences; ***vol.*** *58; nb. 13; (1996); p. 1075 – 1081*

Abstract: Neem seed preparations contain not only azadirachtin as the active insect antifeedant or growth regulator but also a variety of otherlimonoids, some of which are cytotoxic to N1E-115 neuroblastoma (mouse), 143B.TK- osteosarcoma (human) and Sf9 (insect) cultured celllines. The most potent of these limonoids is nimbolide with an IC50 ranging from 4 to 10 µM and averaging 6 µM for the three cell lines. Otherlimonoids of decreasing potency and their average IC50 values (µM) are epoxyazadiradione 27 µM, salannin 112 µM, and nimbin, deacetylnimbinand azadirachtin each > 200 µM (practically nontoxic). Nimbolide at 10 µM acts rapidly in the neuroblastoma cells to induce blebbingassociated with disruption of plasma membranes almost instantaneously and 50percent loss of cell viability within 30 min. At 5 µM nimbolide,the cells become elongated and assume a neuronal shape

accompanied by spikes and lamellipodia within 1-2 hr followed shortly thereafter byextensive cytological changes and vacuolization associated with irreversible processes leading to cell death. Calcium is apparently not involvedin the cytotoxic effect since a calcium-free medium, leading to profound morphological changes, does not alter the sensitivity to nimbolide. Incontrast, epoxyazadiradione requires higher concentrations and a few hr for 50percent viability loss without major morphological changes, indicatinga difference in mode of action for nimbolide and epoxyazadiradione.