CHEMISTRY AND BIOLOGY OF NATURAL PRODUCTS AND THEIR APPLICATIONS FOR HEALTH BENEFITS

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Abstract

The Bio-Organic Division traditionally possesses a rich expertise in organic chemistry that can further be classified into two branches namely natural product chemistry and organic synthesis. Difficult to isolate, low abundant molecules present among a pool of very similar compounds makes the task of isolation of a single compound from natural resources challenging and exciting. Natural product isolation from important plants was done based on their use in Ayurveda or other forms of alternate medicine for different ailments. Biological activity based isolation was also carried out to identify and isolate the active component from a myriad of different molecules. The expertise in synthetic chemistry was exploited to successfully design reactions to synthesise important molecules that were present in minute quantities in natural sources making the isolation process unviable. Combinatorial chemistry approach paved the way for structure function analysis later. This chapter gives the reader the journey/evolution of the biological research over 2 decades that was carried out exploiting the expertise in chemistry and explains chronologically how the two remain intertwined till this date. Thus this chapter explains the contribution of research focussed on different ailments like oxidative stress in chronic

diseases, radiation injury, gastric ulcer, cancer, cardiovascular disease, antibiotic resistance and targeted therapy.

1. Preamble

Modest beginnings of research cooperation between chemistry and biology for health benefits started post 1995 in our Division. The research on natural products to harness their health benefits picked up pace in 1997. Our group was known for its prowess in natural product chemistry and synthetic organic chemistry. The very modest infrastructure i.e. a UV-Vis spectrophotometer coupled with a few reagents and test tubes gave birth to a research programme focussing on translating the expertise in chemistry to biological research focussed on human health related research. Initial period of research i.e. during the period 1997 to 2005 predominantly involved *in vitro* assays assessing the free radical scavenging property/antioxidant property of molecules of diverse nature derived from natural sources mainly botanical in nature. The antioxidant nature of the molecules/plant derived concoctions was investigated with analysis of an extension towards their ability to protect biological macromolecules against gamma radiation or Fenton reaction induced damage. During this period, *in vitro* free radical scavenging assays, assays to evaluate damage to macromolecules like lipid and DNA and kinetic measurements using pulse radiolysis dominated the research methods. Extensive research on Fenton reaction, in extension with the biochemistry of iron and gamma radiation to analyse the ability of different natural products to protect biological targets resulted in deciphering the mechanisms of sacrificial antioxidants or regenerative antioxidants. Some of the natural products that were evaluated for antioxidant and radioprotective properties were *Myristica malabarica*, *Swertia decussata*, *Ginger officinalis*, *Piper betel* etc. Activity based isolation of bioactive molecules from different medicinal plants was carried out in the period between 1997 to 2003.

2. Mechanistic evaluation of the redox properties of natural products

2.1. Folic acid

In regard to studies of antioxidant molecules and their biological relevance, the ability of the physiologically important molecule folic acid to scavenge different free radicals was reported for the first time. Folic acid was seen to scavenge different radicals very efficiently. In the reaction of thiyl radicals with folic acid, it was observed that folic acid not only scavenged thiyl radicals but also repaired thiols at physiological pH. In the lipid peroxidation study, in spite of the fact that folic acid is considerably hydrophilic, it was observed to significantly inhibit microsomal lipid peroxidation. A suitable mechanism for oxidation of folic acid and repair of thiyl radicals by folic acid was proposed after extensive research. Further, due to the interest in the Fenton chemistry, the interaction of folic acid with iron and hydroxyl radicals generated by Fenton reaction was studied. A detailed analysis revealed the participation of freely diffusible hydroxyl radicals in the oxidative degradation of secondary amines including folic acid. Based on direct

evidences employing kinetic measurement techniques, the involvement of a hydrogen abstraction mechanism in the reaction was shown unambiguously. Given the high cellular concentrations of free iron and H_2O_2, it was postulated that the toxic hydroxyl radicals may play a major role in oxidative degradation of folic acid in the cellular systems. Given the multitude of important biochemical reactions in which folic acid is involved in the body, the knowledge emanating from this study was pivotal to evolve strategies to overcome depletion of folic acid and supplementation of it through external means.

2.2. Diketones

In pursuing the interest in the health benefits of bioactive molecules from natural resources, four ginger (*Ginger officinalis*) derived diketones and the popular molecule curcumin from turmeric (*Curcuma longa*) were investigated for their ability to interact with different biologically relevant radicals and protection of macromolecular targets inside the cells. The study revealed the role of additional phenoxy hydroxyl group in curcumin *vis á vis* the diketones from ginger, a reason to exhibit higher activity in protecting against iron mediated lipid damage. The observation that one of the ginger derived diketone, dehydrogingerdione possessed comparable activity to curcumin in iron independent lipid peroxidation assay, lead to the discovery that it had higher affinity to lipid peroxide radical, and possessed superior antioxidant activity compared to physiological antioxidants like vitamins E and C. A synergistic behaviour between the dehydrogingerdione and vitamic C was established, through chemical repair of dehydrogingerdione by vitamin C. Additionally, the study also revealed the important contribution of phenolic and the methylene group in 1,3 diketones in their antioxidant activity.

2.3. Polysaccharides

Diverging from phenolic molecules, research focussing on complex polysaccharides and their ability to protect molecular targets against free radical induced damage was undertaken. An arabinogalactan from *Tinospora cordifolia*, a plant credited with multitude of health benefits was evaluated for its ability to scavenge different radicals and protect lipids and proteins against free radical induced damage. Further, this polysaccharide was shown to protect DNA against gamma radiation induced damage. In order to decipher that it is not a general property of all polysaccharides to exhibit such a protective ability, starch and guar gum were employed to show that the antioxidant and radioprotective properties are unique only to certain polysaccharides. To decipher the contribution of individual monomeric sugars and the type of branching patterns affecting the antioxidative and radioprotective properties in polysaccharides, different polysaccharides were investigated employing radiation protection and antioxidant assays. Employing three different polysaccharides, from three different medicinal plants, our study revealed arabinose, xylose and rhamnose to be major contributors to the antioxidant activity. Interestingly, galactose and mannose did not have any role in the antioxidant activity. The behaviour of one polysaccharide OSP from the medical plant Tulsi (*Ocimum sanctum*) was investigated elaborately due to its differential ability to protect macromolecular targets against iron induced damage vis á vis gamma radiation

induced damage. Our study revealed OSP prevented the deleterious effects of iron by binding to ferric and ferrous ions and rendering them inactive to redox reactions generating free radicals. Later the *in vitro* findings were validated at the cellular level employing mouse fibroblasts cells. The iron induced cell death in fibroblasts was effectively prevented by OSP. During this period multiple medicinal plants were investigated for their biological relevance employing different *in vitro* assays, leading to activity based isolation of individual molecules from them.

2.4. Stilbenes

Stilbenes are a class of phytochemicals that were investigated for different biological activities worldwide. The most important breakthrough discovery in this class of molecules came in 1997, when resveratrol (the well-studied stilbene) was reported for its cancer prevention properties. Due to the low abundance of resveratrol in natural sources like skin of red grapes, peanuts and others, the organic chemists in our group devised novel methods to synthesise stilbenes, notable among which is the Low valent titanium mediated McMurry coupling. A structure activity relationship study on the antioxidant activity of the stilbenes was taken up and in the course of this investigation stilbenes were found to act as DNA damaging agents in presence of metal cations. Mechanistically, this study identified the structural elements required for the DNA cleavage activity, metal ion specificity, and the free radicals involved in the process and generation of double strand breaks in DNA by the stilbenes.

2.5. Modulation of iron by natural products

Fenton reaction is extensively used in free radical research and a variety of modified Fenton reactions gives clue regarding the mechanistic aspects of the molecule under investigation. Extensive analysis of modified Fenton reactions based assays was carried out to investigate the iron modulatory properties of different molecules. Different berberine class of molecules were investigated thoroughly to dissect their ability to prevent free radical induced damage not only due to their ability to scavenge the radicals, but also due to their ability to complex iron, interfere with the redox cycling of iron, thus preventing its ability to induce damaging free radicals. Similarly, a polysaccharide (OSP) from the medicinal plant *Osmium sanctum* was shown to scavenge free radicals generated by Fenton reaction. However, its extraordinary ability to prevent Fenton induced damage could not be explained by free radical scavenging alone. Detailed investigation revealed OSP complexed both ferrous and ferric forms of iron, reduced ferric to ferrous, did not allow ferrous to generate free radicals. Later this interesting property of OSP was extended to cellular level investigations by successfully preventing iron induced fatality to mouse fibroblast cells.

3. Establishment of animal cell culture and a step forward towards cell biology research

In the period starting 2005, the infrastructure to conduct biological research improved with establishment of a cell culture laboratory and obtaining a set of cancer cell lines

from National Cell Culture repository of National Centre for Cell Science, Pune. Further enhancement in capacity occurred when our group procured flow cytometer in 2010 and confocal microscope in 2014. Concomitant to the discovery of pro-oxidant activity of phenolic compounds during the course of investigation on their antioxidant/radioprotective property provided a genesis towards exploitation of such a property towards killing cancer cells. Once the free radical chemistry behind the action of different molecules was evaluated, with the improvement in the facilities to biological research, the findings were extended to biological systems especially cancer cells in culture. The sensitization of cancer cells by natural products, induction of cell death in cancer cells and the mechanisms underlying such process took a foot hold post 2005. In this period detailed analysis of the free radical chemistry behind berberine, bakuchiol, coralyne etc. was also taken up. It was established that coralyne, synthetic congener of the natural protoberberine alkaloid berberine, possesses DNA photonicking property. Extending the findings, biological studies revealed that compared to coralyne alone, coralyne and UV-A (termed CUVA) efficiently killed cancer cells irrespective of the p53 status of the target cells. Further studies also revealed, in association with UV-A, coralyne, but not related molecules berberine and jatrorrhizine induced significant nicking of plasmid DNA *via* an Oxygen-independent photo-chemical processes. The DNA photo-nicking by the combination of CUVA was primarily caused by the coralyne aggregates without any significant contribution from the DNA-intercalated coralyne monomer. The DNA damaging property of stilbenes which was established *in vitro* was extended to cell culture studies to evaluate their anti-cancer property and the role of DNA damage.

4. In vivo studies to cure ulcer through natural products

Parallel to the research on the free radicals, our work also indulged in evaluating the health benefits of natural products in terms of protecting against NSAID induced adverse effects especially the gastric ulcer. Under the group of such investigations, the following class of molecules were evaluated namely stilbenes, malabaricones, catechins etc. employing mouse models of research. Expertise to handle small rodents like mice and rats in the animal house facility was developed. Withdrawal of blood, excision of tissues from different organs for histopathological and immunological analysis to understand the progress of disease/prognosis after treatment in animal models was a remarkable path forward in terms of capacity building. Notable achievements in this period are healing of indomethacin induced stomach ulcers by epigallocatechin gallate and establishment of the role of COX-independent pathways in this process. On the same lines investigations on the ulcer healing properties of black tea, theaflavins, stilbenes and *Piper betel* derived allylpyrocatechol were carried out and published.

As part of evaluating the healing properties of natural products, we developed an incisional wound model in rats to the evaluate wound healing capability *Piper betel* extract. We prepared various formulations of Piper betel extract (in paraffin oil, petroleum jelly and hydroxyethyl cellulose) and evaluated their ability to heal excisional

wounds in rats. We found that a formulation containing 5 % crude Piper betel extract in a 2 % hydroxyethyl cellulose base gave significantly superior wound healing activity compared to soframycin. In order to determine the principal active component responsible for wound healing, we prepared crude Piper betel extracts depleted of 4-allyl-pyrocatechol (APC, the principal active component of Piper betel extracts as reported previously by our laboratory and others). The depleted extract showed no significant wound healing activity, thus establishing APC as the principal component responsible for wound healing activity of Piper betel extract.

5. Role of organic chemistry and its contributions to biological research

Extensive contributions by the natural product chemists who isolated active molecules from bulk quantities of plants by solvent extraction formed the backbone of the natural products research. Considering the low natural abundance of different bioactive molecules identified by the research carried out with the focus on health benefits, expertise in synthetic organic chemistry was brought to the fore for the efficient synthesis of selected molecules for further evaluation. The cost of isolation of these natural molecules from their parent sources is cumbersome, environmentally unfriendly and are not cost effective. Further due to the similar nature of the molecules, it was technically challenging to isolate them from natural resources where they are present as a cocktail. Hence it was prudent to device methods using synthetic organic chemistry to obtain them in larger quantities from simple precursors using clever chemistry.

5.1. Synthesis of malabaricones:

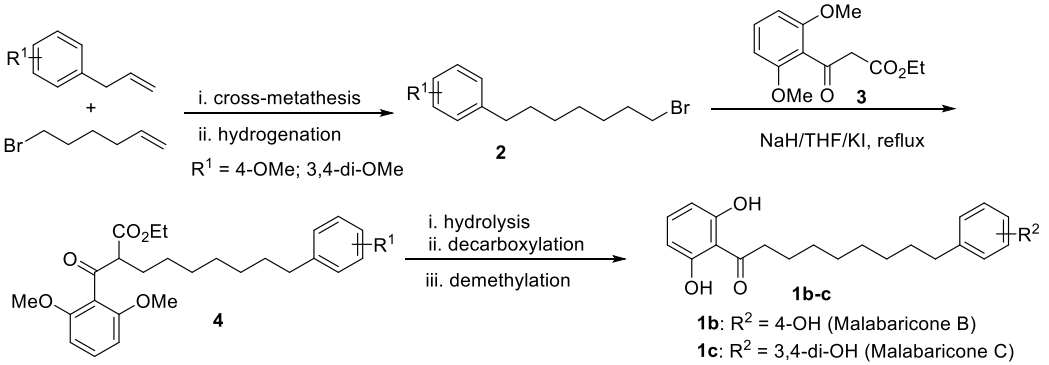


Fig. 1: Complete synthesis of low abundance natural products malabaricone B and malabaricone C via cross-metathesis strategy (Journal of Natural Products 2017, 80, 1776)

To this end, a novel, efficient and practical route for synthesis of malabaricones was developed. Olefin cross-metathesis strategy was successfully utilized as the key steps for total synthesis of malabaricone B and C. The ω-arylheptylbromide, which is one of the key building blocks of the synthetic protocol was achieved via successful cross-metathesis reaction of allylarenes and 6-bromo-1-hexene followed by chemoselective

hydrogenation of double bond in presence of bromide. 2,6-dimethoxyphenyl-β-keto ester was then subjected to successful C-alkylation reaction with ω-arylheptylbromide, in presence of sodium hydride as base and potassium iodide (KI) as additive to afford the corresponding β-keto ester as the product. Malabaricone B and malabaricone C were then obtained by subsequent alkaline hydrolysis, *in-situ* decarboxylation and demethylation. The bio-activities of the synthesized compounds were found to be similar with the compounds isolated from natural product. This synthetic protocol was employed towards total synthesis of all other member of the malabaricone family including malabaricone A and malabaricone D. The methodology developed has various advantages, such as higher yield of product, minimum number of steps and simpler reaction conditions.

5.2. Synthesis of stilbenes

Similar to malabaricones, hydroxystilbenes also occur naturally in several plants in very low amounts. Detailed biological evaluation of promising hydroxystilbenes as well as structure function studies of such molecules was hampered due to the challenges in isolating them as mentioned above. To meet the need of various hydroxystilbene derivatives towards different biological applications, an easy and convenient protocol for synthesis of this important class of molecules was established. Using Low valent titanium (LVT) reagents to mediate reductive coupling of two carbonyl compounds was supposed to be the most convenient way to achieve this important class of molecules. However, when suitably substituted phenolic aldehydes were reacted with LVT reagent [TiCl3-Zn-THF] desired hydroxystilbenes were formed along with corresponding dihydro-compounds, by in-situ reduction of the stilbenic double bond. In contrast, it was shown that when phenolic ketones were used as substrates, stilbene compounds were formed as the sole product. This work established that phenolic aldehydes are not suitable starting materials to obtain hydroxystilbenes by LVT mediated reactions. Since, use of free hydroxyl-group promoted in-situ-hydrogenation of the product methoxy-substituted benzaldehydes were used as the starting materials in this LVT method to obtain corresponding methoxy-stilbenes, which were subsequently de-methylated to their hydroxy-counterparts by using BBr3. Thus, our research proved that LVT promoted method could give easy access to biologically important polyphenolic stilbenes in two steps.

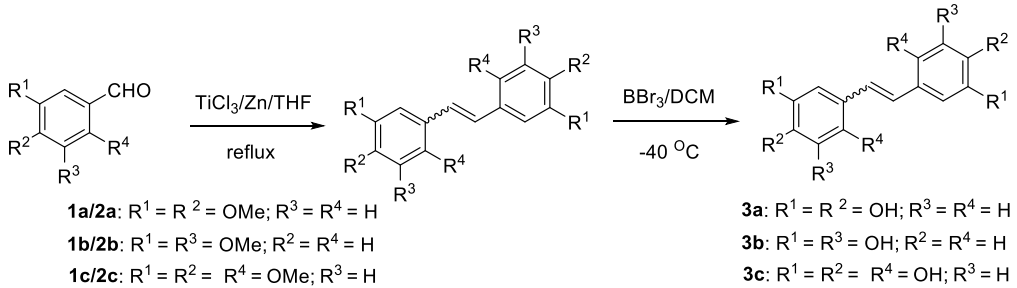
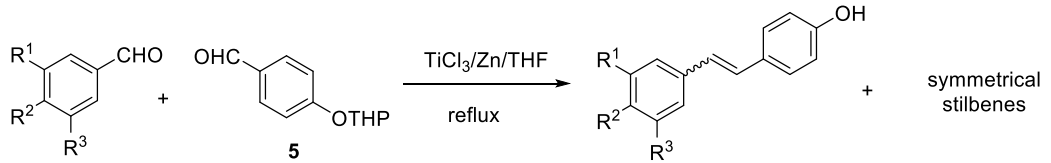


Fig. 2: Synthesis of hydroxystilbenes from methoxy-substituted benzaldehydes employing low valent titanium method (Indian Journal of Chemistry B 2004, 30, 1934)

Along with polyhydroxy stilbenes, many partially methylated hydroxystilbenes also possess important pharmacological activities but it was realized that above method was unsuitable for synthesis of this class of molecules. Due to the interest in LVT reagents, several modifications of this reagent were formulated by our research group to perform different organic transformations which were earlier not reported. Utilizing this understanding, an alternative synthetic route to access partially methylated hydroxystilbenes by using tetrahydropyranyl (THP) protected phenolic benzaldehydes was successfully developed. Here, methoxy substituted phenolic benzaldehydes/phenolic benzaldehydes were initially pyranylated, followed by LVT mediated reductive coupling in presence of [TiCl3-Zn-THF]. During this process, in-situ removal of the THP group occurred while the methoxy group remained intact to provide the corresponding partially methylated hydroxystilbenes. Thus, a library of different stilbene molecules was synthesised successfully to purity. These molecules were evaluated for different biological activities and structure-function relationship studies.



4a: $R^1 = R^3 = OMe$; $R^2 = H$

4b: $R^1 = OMe$; $R^2 = OTHP$; $R^3 = H$

6a: $R^1 = R^3 = OMe$; $R^2 = H$

6b: $R^1 = OMe$; $R^2 = OH$; $R^3 = H$

Fig. 3. Synthesis of partially methylated hydroxystilbenes employing low valent titanium method. (Indian Journal of Chemistry B 2004, 30, 1934)

5.3. Synthesis of conjugated natural molecules for targeting cellular organelles in cancer

Apart from the above-mentioned success stories where organic chemistry and cell biology held hands together to further the quality of research, recent demands to test novel ideas provided a platform for collaboration. Intense research in biology employing cancer cells revealed the molecular targets of selected natural products/synthetic congeners. We hypothesised that targeting these molecules to specific compartment of a cancer cells would increase the efficacy by several folds. The challenge provided to the organic chemists was to combine two molecules into one i.e. molecule A (targeting molecule) that carries molecule B (the drug) to a specific organelle inside the cancer cell so that efficient killing of the cancer cell occurs. In this regard, we synthesised mitochondria targeting stilbenes and malabaricones, which showed 10-20 fold higher efficacy in killing cancer cells in various preclinical models. For the first time, a hypothesis driven design and synthesis of lysosome targeting stilbenes and endoplasmic targeting BOIDPYs was carried out, that is specifically effective against different forms of pancreatic cancer. It may be noted that pancreatic cancers are very difficult to treat and exhibit a high rate of fatality. The continuous effort in this direction has allowed us to

establish the molecular basis of sensitization of pancreatic and other cancers through organelle targeting.

The molecules thus synthesised and characterized as pure were employed in structure-function studies, pharmacological profiling, animal studies and decrease the cost of obtaining them in enantiomeric purity. The valuable contributions from collaborating partners i.e. organic chemists and cell biologists thus resulted in testing new hypotheses that led to good quality research.

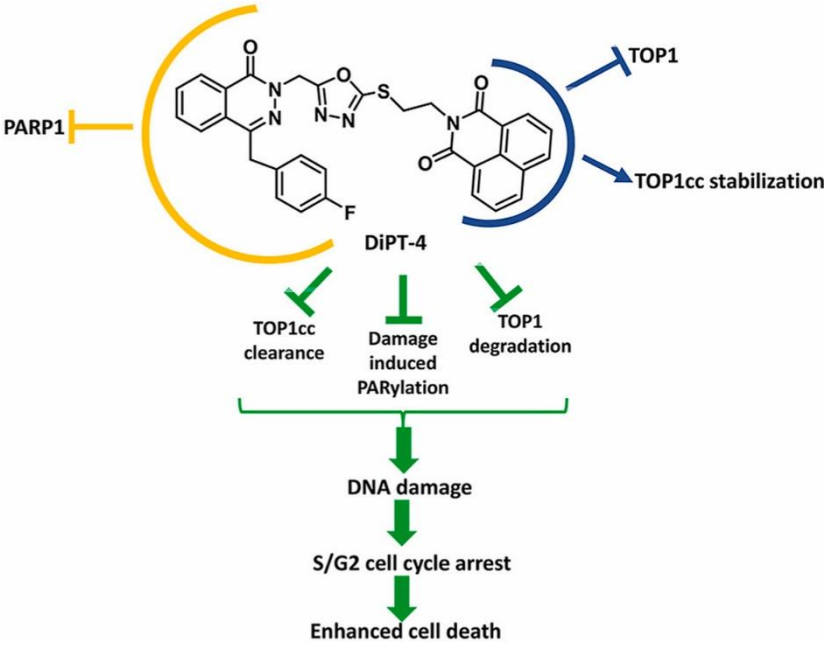


Fig. 4: Design, synthesis and development of a dual inhibitor of Topoisomerase 1 and poly (ADP-ribose) polymerase 1 [PARP1] for efficient killing of cancer cells (European Journal of Medicinal Chemistry 2023, 258, 115598)

6. Combating antibiotic resistant bacteria employing natural products

Considering the potential of phenolic compounds to act as helper compounds or as standalone drugs to counter antibiotic resistant bacteria, resveratrol was established as an antibacterial molecule. Contrary to the popular belief in the existing literature, research from our group found no role of diffusible reactive oxygen species in the antibacterial activity of resveratrol. Further, we established DNA damage is a late event in the bacterial cell death induced by resveratrol. A comprehensive structure function correlation encompassing a multitude of parameters established important structural elements that are mandatory for a successful antibacterial stilbene. Dimer stilbene (DS) was found to be a superior antibacterial molecule than resveratrol in this study. Further studies on the antibacterial potential of DS revealed that it synergizes with antibiotics that target protein synthesis. Detailed analysis employing different stilbenes revealed this

property of synergizing with antibiotics targeting protein synthesis is not unique to DS alone, but is a common property among antibacterial stilbenes. The proof of concept experiment was also demonstrated in an animal infection model employing Swiss mice. In a different study on this topic, hydroxychavicol derived from *Piper betel* was shown to damage Fe-S proteins, engage in redox cycling of oxygen radicals, damage bacterial membrane and DNA leading to cell death in bacterial cells. A time kinetics revealed hydroxychavicol induces oxidative stress, membrane damage and DNA damage in that order. Finally, the membrane damage induced by hydroxychavicol results in entry of hydrophobic antibiotics into difficult to treat gram negative bacteria, augmenting our arsenal against antibiotic resistant bacteria.

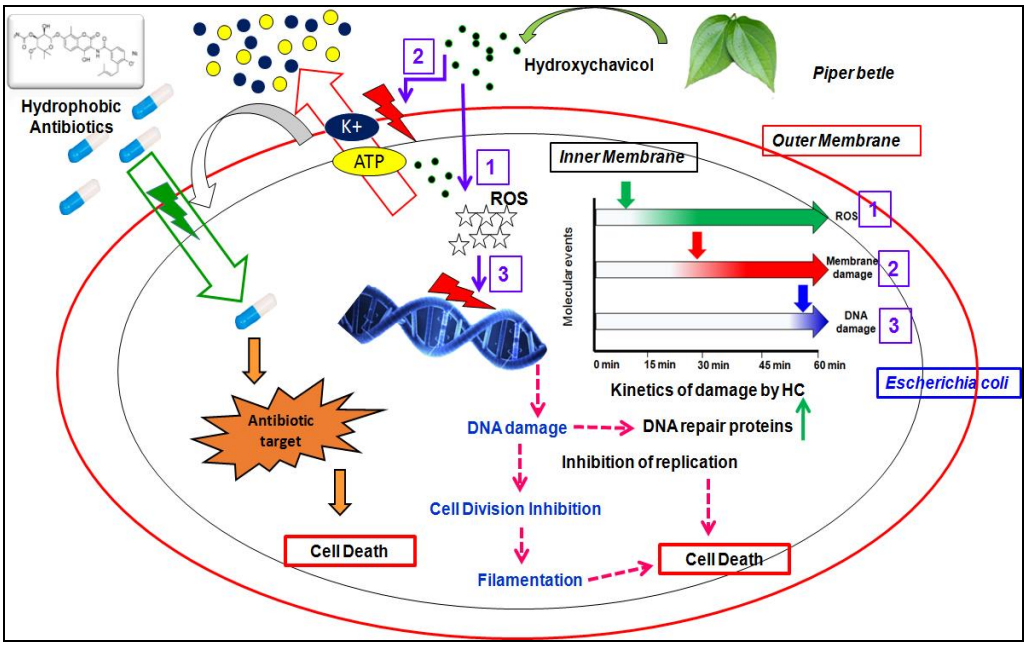


Fig. 5: Hydroxychavicol derived from *Piper betel* leaves induces ROS mediated macromolecular damage in bacterial cells and aids entry of hydrophobic antibiotics into bacterial cells leading to killing of antibiotic resistant bacteria (Biochimie 2021, 180, 158)

7. Improving cardiovascular health through phytochemicals

Cardiovascular disease (CVD) is a disease of heart, kidney, brain and blood vessels, accounting for 1.5 million deaths annually in India. Chronic hypertension (HT) is the insidious culprit and a major risk factors of CVD prevalence. Uncontrolled HT leads to a pathological process to the heart and vasculature, known as cardiovascular remodelling that involves change in the size, shape and function of the heart and vessels. We evaluated the ability of the natural molecule allylpyrocatechol (APC) isolated from Piper betel to remodulate cardiovascular properties in a volume overload, high salt model of hypertension in rats. APC (10 mg/kg b wt) significantly attenuates hypertension in rats. It

corrected the atrial electrical conduction irregularities as well improving the ventricular contractility and pumping efficiency. It reduced organ hypertrophy and fibrosis improving the cardiac and renal functions. In addition, it improved the smooth muscle functions of aorta thus improving its vasoreactivity in response to increased haemodynamic load.

In another study, malabaricone C, another natural product exhibited excellent antihypertensive and antihypertrophic activity superior to curcumin. In addition, Mal C improved the vascular flow and vascular reactivity by protecting the endothelial layer of the blood vessel reducing its oxidative damage. It also reduced cardiac, adrenal and renal hypertrophy indicating better organ functions. It also helped in depolarization potential of cardiomyocytes and papillary muscles indicating significantly better electric profile, contractility, valve function and pumping function. It reduced potent vasoconstrictors like endothelin and anti-diuretic hormone like vasopressin leading to reduced haemodynamic load. It brought down the organ and blood oxidative stress levels. Thus, our studies in animal models proved the potential benefits of natural products mentioned above in improving heart health.

8. Upsetting the redox balance in cancer to induce cell death

Recognizing the importance of the skewed redox balance in cancer cells compared to non-cancerous cells, we hypothesised that any molecule that could disturb the antioxidant- prooxidant balance in cancer cells would potentially be cytotoxic to them. Considering the ability of some natural products from botanical sources like malabaricones to modulate the redox balance inside cells, it was postulated that these molecules could be cytotoxic to cancer cells while sparing the normal cells. Malabaricone C (mal C), a promising molecule from the group of malabaricones isolated from the plant *Myristica malabarica* was capable of inducing oxidative DNA damage leading to cell death in different cancer cell lines. Mal C induced single strand breaks as well as the lethal double strand breaks in the DNA followed by p38-MAPK activation, imbalance in BAX/BCL2 ratio leading to mitochondrial dysfunction in lung cancer cells. Intriguingly, in the breast cancer cells treated with mal C intracellular Ca^{2+} release, calpain activation, lysosomal membrane permeabilization (LMP) were found to be the critical events leading to cell death. In another interesting piece of work, mal C treated cancer cells exhibited higher amount of intracellular reactive oxygen species (ROS) while addition of thiol antioxidants sensitized the cancer cells to death instead of protecting them. This apparent anomaly was solved by proving that thiol antioxidants recycled mal C from oxidized to reduced state and generated more and more site specific ROS in the process. Additionally, the thiol antioxidants also lead to S-glutathionylation of key transcription factors (p53 and p65) leading to abrogation of their protective role against ROS induced cell death. We also showed that the sister molecule malabaricone B (mal B) induced cell death in cancer cells of different tissue origins, independent of the p53 status in them. It is also important to note that mal C and mal B were nontoxic to normal cells at the concentrations that were cytotoxic to cancer cells. Based on the

promising results on the work on malabaricones, recently mal C was tagged to triphenyl phosphine to target it to mitochondria. Targeting promising anticancer molecules to specific organelles inside cancer cells is expected to increase the efficacy of these molecules.

Investigation of the redox active hydroxychavicol isolated from *Piper betel* against pancreatic cancer revealed, this not only induces extensive DNA damage but also forces the cells into mitotic catastrophe. The cell death by hydroxychavicol was induced by JNK pathway-dependent, caspase-mediated apoptosis. Hydroxychavicol also inhibits migration and invasion of pancreatic cancer cells via a generalized repression of genes involved in endothelial mesenchymal transition.

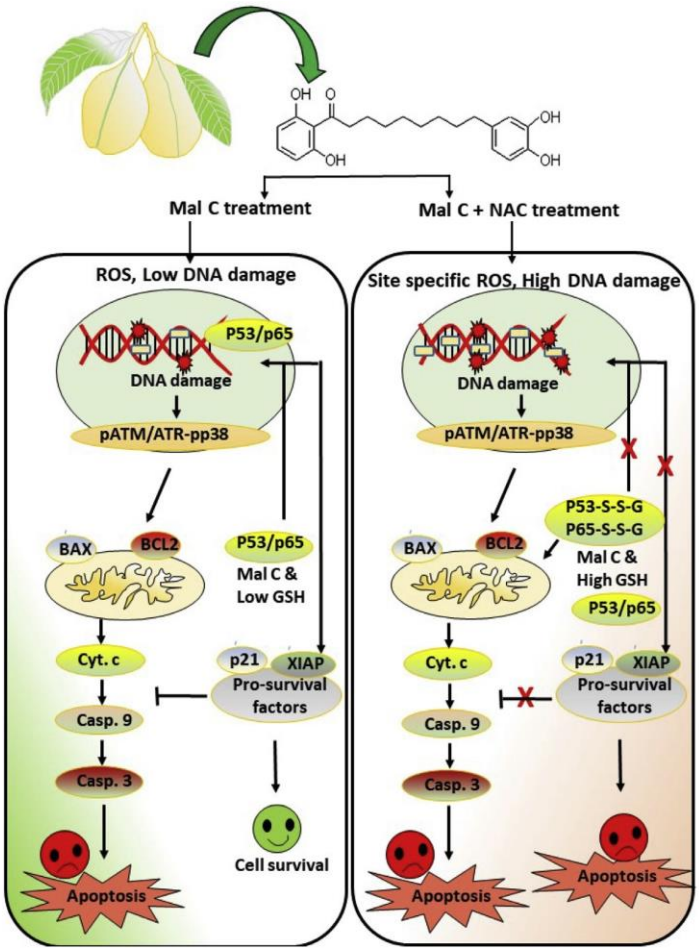


Fig. 6: Natural product malabaricone C reprogrammes redox sensitive proteins p53 and NF-κB in response to thiol antioxidants and induces cancer cell death in vitro and in vivo (Free Radical Biology and Medicine 2020, 148,182)

9. Understanding the fundamental processes in cancer cells and exploiting its Achilles heel

Cancer cells fundamentally differ from a normal cell in many ways. Key differences that could determine the fate of the cancer cell's survival include rapid replication of the genome, cell cycle check points, repairing the damages to the DNA etc. Due to mutations in some of the pathways, the cancer cells are more reliant on/addicted to alternate pathways for their survival. We realised that it is important to unravel such addictions of the cancer cell and induce damages that is repaired by the pathway carrying the mutation. At the same time if the alternate pathway is also blocked by an inhibitor the damage cannot be repaired and results in cell death. This process is called "synthetic lethality". Diverse examples of this concept exist that have the potential to keep different cancers at bay substantiated by a few examples below.

In a study to overcome the limited use of PARP inhibitors in cancer therapy due to their inability to work well in homologous recombination (HR) proficient cancers, we postulated the use of stilbene resveratrol as a chemosensitizer. The resistance to PARP inhibitor in cancers is, at least in part, due to activation of autophagy. Considering resveratrol is a modulator of autophagy, we evaluated the mechanisms behind the ability of resveratrol to enhance the efficacy of PARP inhibitors. Our work established resveratrol induced dysregulation of cell cycle and enhanced PARP inhibitor talazoparib -induced double strand breaks (DSBs), leading to mitotic catastrophe. We also found that resveratrol attenuated fusion of autophagosome and lysosome though induction of lysosomal-membrane-permeabilization (LMP) preventing autophagy and overcoming resistance to PARP inhibitors. Our investigation on the efficacy of different stilbenes *vis-a-vis* resveratrol as potential anti-cancer agents resulted in the identification of dihydroxystilbene (DHS) as a more potent anti-cancer agent than resveratrol. This was established using neuroblastoma tumor model and a melanoma model in mice.

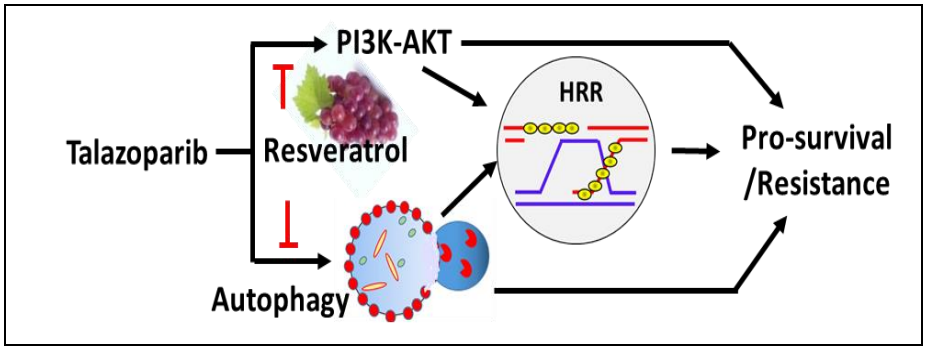


Fig. 7: Natural product resveratrol overcomes resistance to anticancer drug talazoparib by inhibiting participation of talazoparib induced autophagy and PI3K-AKT in homologous recombination repair pathway (Biochemical Pharmacology 2022, 199, 115024)

Replication stress is a phenomenon defined as the state of cells when the entire genome is unable to be replicated and as a consequence the progress of the cells through S phase is impaired. It has been reported previously that stilbenes like resveratrol cause replication stress. We observed PARP inhibitors and stilbenes (for e.g. DHS) induce replication stress individually in cancer cells. However, when combined, these two (talazoparib and DHS) molecules acted synergistically where in the cancer cells exhibited significantly higher DSBs, incurred through extensive damage at replication forks. The cells treated with the combination spent protracted time in S phase and are unable to overcome the replication stress, triggering replication catastrophe and ultimately cell death. The proof of this concept was also successfully demonstrated in ovarian cancer model in SCID mice.

Post 2020, our group has devoted its resources completely on research on cancer especially in its response to radiation, radiopharmaceuticals, chemotherapeutics to develop targeted therapeutics and precision medicines. Mechanistically, research is heavily focussed on understanding the differences that exist in fundamental molecular processes like DNA damage repair pathways, replication stress, autophagy, mitochondria-lysosome homeostasis, cell cycle defects between a cancer cell and normal cell. The thorough understanding emanating from such studies help us employ "targeted-natural products" that interfere with such processes and kill cancer cells. Successful molecules/combinations thus identified are tested on appropriate preclinical models to establish the efficacy of the treatment.

10. Conclusion

As explained above, the research in our group was predominantly Chemistry and Biology of free radicals prior to 2000. In the next two decades, our objectives to exploit phytochemistry to augment health benefits in terms of disease cure made progress in leaps and bounds in addressing diverse diseases like cancer, cardiovascular disease, gastro intestinal ulcer, antibiotic resistant bacterial infections to mention a few. Remarkable feats were achieved in terms of understanding diseases and identification of potential drugs to cure them. True to the spirit of scientific pursuit, the outcomes of the research are credited to the team work especially the diverse expertise brought to the table by "organic chemists" and "biologists" – by "Bio-Organic Researchers". The scientific programme that has undergone the evolution as explained above continues to work on human health related aspects, augmenting the research with understanding fundamental processes inside a cell. This basic science driven research forms a main pillar towards developing strategies/drugs to combat different diseases.

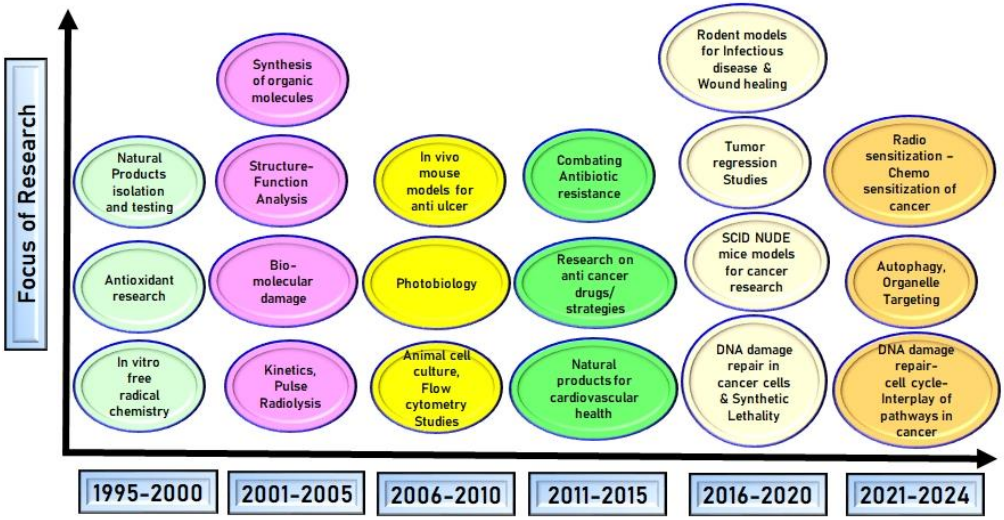


Fig. 8: The journey depicting evolution of scientific work between 1995 to 2024

11. Acknowledgements

The authors wish to acknowledge the contributions of the scientists to the above mentioned research programmes, whose unwavering enthusiasm, hard work, foresight, creativity, perseverance and leadership resulted in successful achievement of the objectives.

Dr. Ashoke Banerjee, Dr. Subrata Chattopadhyay, Dr. Sandip Kumar Nayak, Dr. Sunil Ghosh, Dr. Tapan Kumar Ghanty, Dr. Gajanand Chintalwar, Dr. Hassranjani, Dr. Birija Sankar Patro, Dr. Soumyakanti Adhikary, Dr. Mahesh Subramanian, Dr. Soumyaditya Mula, Dr. Dibakar Goswami, Dr. Ajoy Kumar Bauri, Dr. Jitesh Singh Rathee, Dr. Mrityunjay Tyagi, Dr. Kshama Kundu, Dr. Papiya Dey, Dr. Saikat Chakraborty, Dr. Pooja Gupta, Dr. Sunita Gamre, Mr. Ganesh Pai, Mr. Ananda Guha Majumdar, Mr. Nitish Chauhan, Mr. Sahil Kumar, and Ms. Riddhi Pal.