Discovery of Drugs

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It is a moment of joy to write this article to commemorate the milestone of 100 issues of **CuttingEdge**. *In my journey into discovery of drugs, I was fortunate to have used a galaxy of Shimadzu HPLC machines. I believe that Machines are great teachers. I was fortunate also because of the professional, friendly and educational interactions I had with a large number of Service Engineers from Spinco Biotech. Further, as a platform for refreshing* **CuttingEdge** *news about the how and why of research tools and technologies,* **CuttingEdge** *has been a boon for researchers across India. It gives the feeling of a large family of practitioners of Science wanting to design and perform innovative experiments to create new Science. The magazine enables researchers to ask questions and keep learning all the time. Thus through the article by Dr. Venkat Manohar on UHPLC in the latest issue of* **CuttingEdge**, *I have become familiar with the beauty of UHPLC and I look at it as a welcome addition in the evolution of chromatography. My congratulations to* **CuttingEdge** *for the magnificent century and best wishes for healthy growth in all domains of enterprise.*

Misery has its origins in sick thought and sick body. Further mind and body are so intertwined (*I think therefore I am*) that one can easily influence the other. Drugs are meant to cure both these kinds of miseries. However sometimes drugs cure only those who wish to be cured and do not cure the ones who do not wish to be cured. Also an inert substance given as a drug can bring relief (Placebo) or bring misery (Nocebo) in different individuals under different circumstances. Drugs must be safe, be effective in miniscule amounts, have modes of delivery that are convenient, should have no side effects and of course must be affordable. When about fifteen years ago as a biochemist plus Peptide and Protein chemist, I ventured into Drug Discovery against Malaria I was fascinated by the very nature of drugs and all the challenges of discovering drugs. Very soon I had learnt that most successful drugs like Morphine, Quinine, Artemisinin, Paclitaxel, Digitalis, Aspirin, Vincristine & Vinblastine are derived from plants. As depicted in Fig. 1, I was inspired a lot by the question. Why most drugs are Nature derived molecules while most compounds synthesized by chemical combinatorial routes are not drugs?

I was inspired also by Jon Clardy who suggested that it may be because while Nature's specialized metabolites

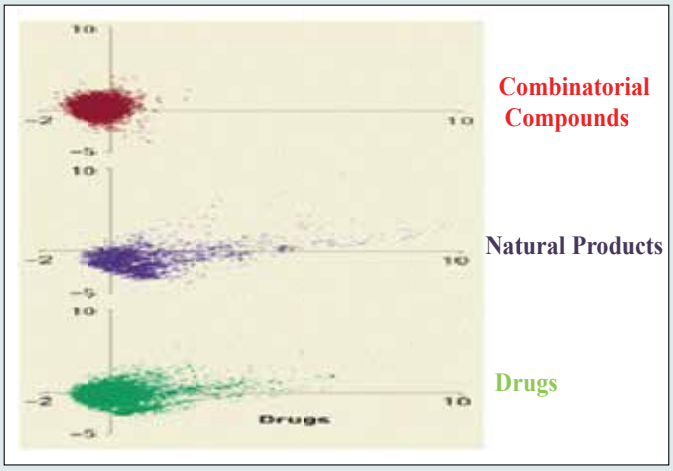

Fig 1: Chemical space diagram showing Combinatorial compounds densely populate a small area, whereas natural products are more spread out.

have come up after biological evolutionary history of millions of years all along rubbing shoulders with biological niches including membranes, proteins and nucleic acids, the chemically synthesized combinatorial compounds are 'inexperienced' in dealing with biology. In the background of this perspective and in collaboration with Dr.Padmakumar of the University of Kerala, my laboratory at the ICGEB, New Delhi started exploring marine organisms with the aim of discovering Novel drugs against malaria. Our companions in this venture were *in vitro* culture of *Plasmodium falciparum* in human red blood cells (Fig 2) and Chromatographic separations driven antiplasmodial activity guided isolation of pure compounds. The redeeming feature of our high throughput growth inhibition assay (Fig. 2) was that the enucleate phenotype of human red blood cells allowed us to use SYBR Green fluorescence to monitor the growth of malaria parasite in culture.

Towards Structure determination of isolated antiplasmodial compounds (Figs 3 - 5) we had excellent collaboration with Dr. Madhav Sharma and his group at IICT, Hyderabad. For example, what appeared to be a pure sample (Fig. 6) was on the basis of NMR suspected to be a mixture of two molecules.

When we made some chemical changes (e.g Switching from Methanol-Water to Acetonitrile - water gradient) in the RPHPLC system it was heartening to see that the single peak (Fig. 6) gave rise to two distinct well resolved

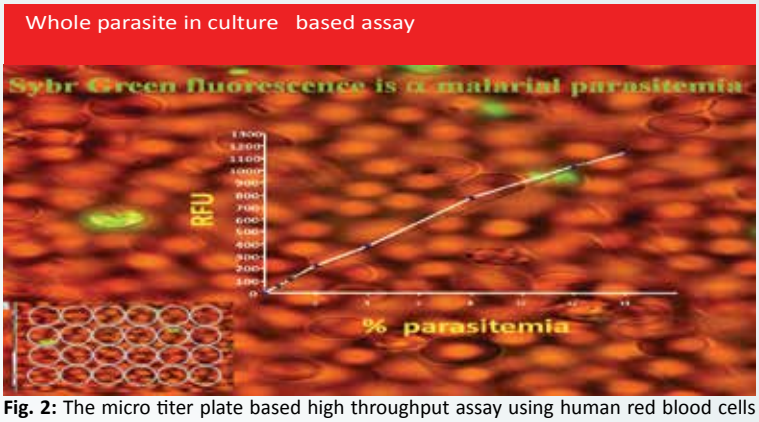

Fig. 2: The micro titer plate based high throughput assay using human red blood cells allows the use of SYBR Green fluorescence to monitor the growth of blood stage malaria parasite.

Figure 3 shows the preparative scale RPHPLC separation and biological analysis of one of the extracts (178) we studied:

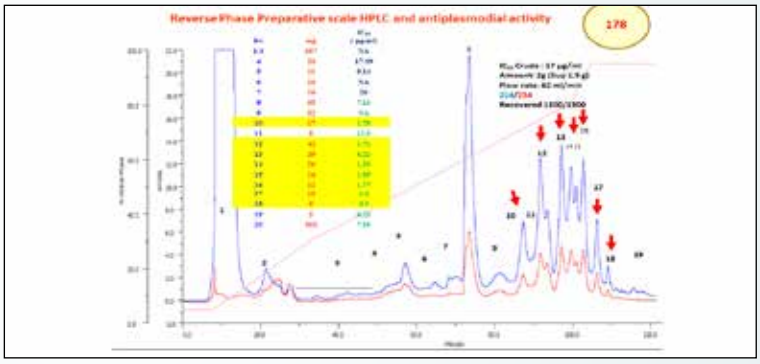

Fig. 3: RPHPLC Separation of specialized metabolites obtained from a marine organism. Numbers in black represent fractions collected and table indicates antiplasmodial IC50 for each fraction. Red arrows indicate promising fractions. Notable is the rise in potency in going from crude (IC_{50} 17 µg/ml) to some fractions with IC_{50} ~ 1 µg/ml.

Fig 4 shows further purification of fraction 10 using semipreparative and analytical modes of RPHPLC Purification.

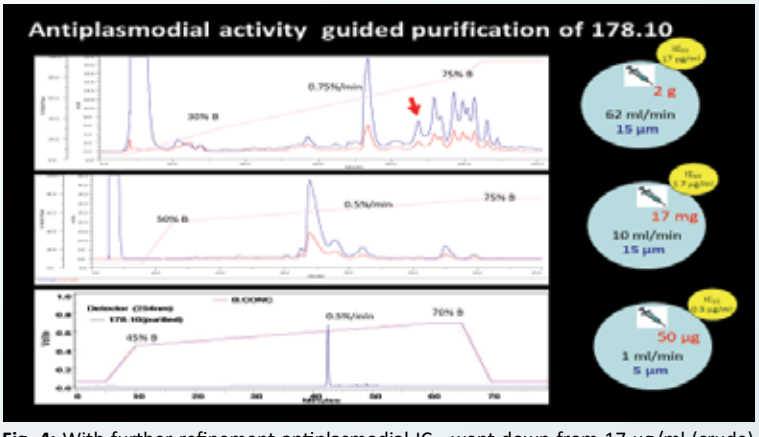

Fig. 4: With further refinement antiplasmodial IC_{50} went down from 17 µg/ml (crude) to 1.7 µg /ml (partially purified) to 0.5 µg /ml (highly purified). Note the changing gradients, flow rates, particle sizes of beads used and amounts of samples loaded.

Fig 5 shows the large number of molecules with promise against malaria we purified from 178.

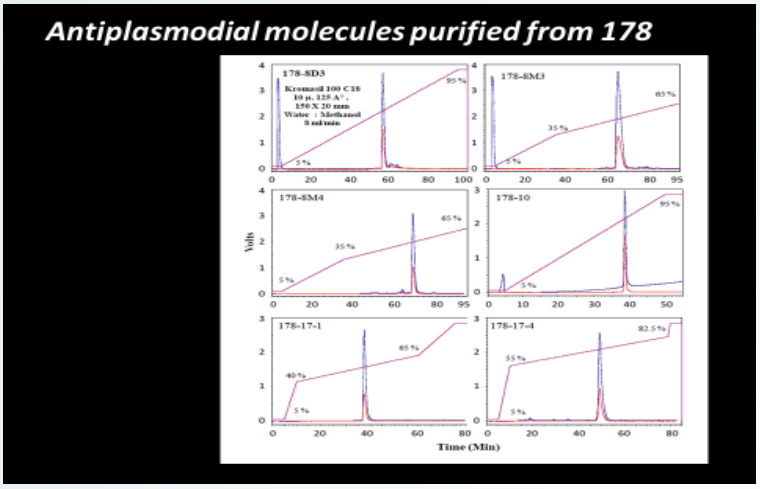

Fig. 5: A galaxy of purified antiplasmodial molecules from 178

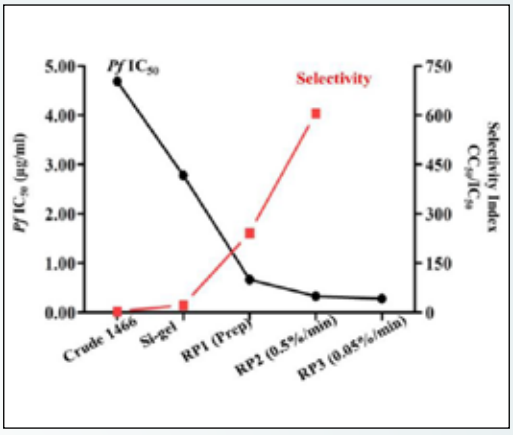

Fig. 8: Chromatographic purification has in it the power to potentiate a drug and to enhance its selectivity.

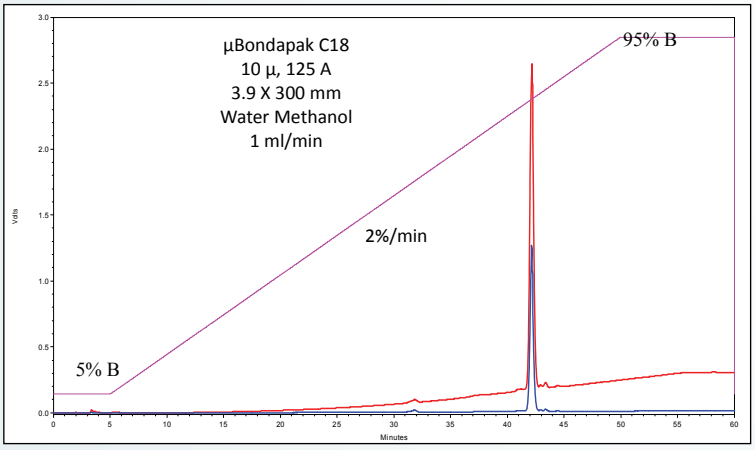

Fig. 6: RPHPLC profile of a pure looking molecule that turned out to be a mixture of two molecules.

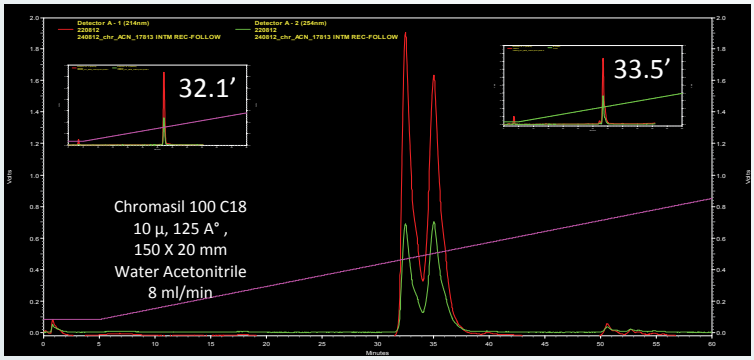

Fig. 7: The single peak of Fig 6 was resolved into two distinct peaks using a new set of solvents and a new gradient. Insets on left and right show the purity of the collected molecules with their respective retention times.

peaks (Fig. 7). When the chemical structures were unearthed it turned out that the two molecules were structurally close.

In other projects we explored the antiplasmodial potential of medicinal plants from India, Nigeria and Cameroon. More recently, in collaboration with Dr.T.S.Suryanarayanan, Vivekananda Institute of Tropical Mycology (VINSTROM) Chennai we are engaged in exploring the promise against malaria of non-sporulating endophytic fungi obtained from the plants of Andaman Islands. Antiplasmodial activity guided chromatographic purification on XAD trapped secretome of one promising endophytic fungus has led to 17-fold increase in antiplasmodial potency {from IC_{50} 4.69 µg/ml (crude) to 0.28 µg/ml (purified)} concomitant with >190-fold increase in selectivity (Fig. 8).

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