Searching for new chemical entities with automated bioactivity-guided cell-based assays

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The Nicholas Piramal Research Centre (NPRC) is the research wing of the pharmaceutical company, Nicholas Piramal India Limited. In 2005, a compound screening facility was established for the NPRC in Mumbai, India, to accelerate identification of lead compounds for drug discovery from natural products and small molecule libraries. The facility depends on a Freedom EVO® 200 liquid handling workstation to automate a wide range of biochemical and cell-based assays that identify candidate compounds for the treatment of cancers, metabolic diseases and inflammatory disorders. This is one of the first fully functional, integrated screening facilities in India.

The center's Freedom EVO 200 is equipped with a Te-MO™ 96-channel pipetting head, an 8-channel liquid handling (LiHa) arm and a robotic manipulator (RoMa) arm. A Safire²™ microplate reader and 96 PW™ microplate power washer are also integrated with the platform, along with third party instruments that include a Cytomat® (from Heraeus) and centrifuge (from Hettich). This automated set-up was chosen to increase the throughput rate of cell-based end point assays, receptor ligand binding studies, kinase assays, protease assays, molecular interaction assays and sample library generation. The platform is programmed to perform the assays using Tecan's Freedom EVOware® Plus software, which combines pipetting and scheduling in a single, scalable package. Being able to automate all the assays using a variety of different cell lines with a single platform was a critical factor when deciding which robot to choose. The Tecan platform allows simultaneous testing of hundreds of compounds in triplicate against many assays relevant to diseases such as cancers, inflammatory and metabolic disorders. The Safire²'s multimodality makes it suitable for automatically measuring many different assay endpoints, including absorbance, fluorescence, chemiluminescence, fluorescence polarization, FRET (fluorescence resonance energy transfer) and other modes.

Automated screening of natural product libraries

Chemical substances derived from animals, plants and microbes have been used to treat human disease since the dawn of medicine. This tradition is the basis of alternative medicine as practised in Indian culture, eg. ayurveda, siddha, unani, homeopathy, etc. Investigating natural products as a source of novel human therapeutics is one of the major objectives of high throughput screening at NPRC, and an assortment of sample libraries consisting of unique, unrelated natural and synthetic compounds are screened for leads. Specific libraries are assayed across different disease targets and, when a positive lead is obtained, this is subjected to bioactivity-based fractionation until the novel molecule is identified and structurally elucidated. Sources of interest at the NPRC include herbal, marine, actinomycetes and fungal products, which are screened with disease relevant phenotypic assays to prioritize and isolate compounds or extracts with anti-cancer, anti-inflammatory and anti-diabetic potential.

From left to right: Amit Khanna, Prabakar Sampath, Prabhu Padmanabhan, Arun Srinivasan, Hitesh Goswami, Giridharan Periyasamy, Balakrishna Shankar, Arun Balakrishnan, Asha Almeida

Bioactivity-based fractionation

The bioactivity of the natural product extract is validated using cell-based assays that have been set up in-house as a primary screen for each therapeutic area. The primary screen is repeated many times to further localize and isolate the active ingredient. These prioritized compounds are then put through secondary screens that are specifically designed and established for individual disease targets, allowing us to postulate the novel molecule's possible mechanism of action. At present, our throughput using the Freedom EVO allows us to generate around 10,000 data points per week. The workstation is used continuously to run multiple experiments in a single day and our screening strategy is novel in profiling the leads across all disease targets to define the effect of the molecule, prior to its inclusion for animal studies.

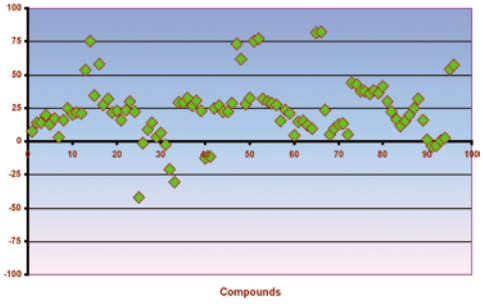
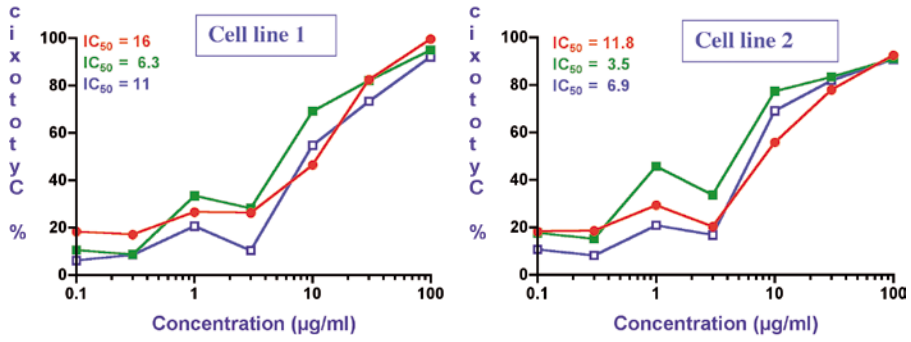


Figure 1:
Screening output of an anticancer screen using natural products from multiple sources on the high throughput screening system



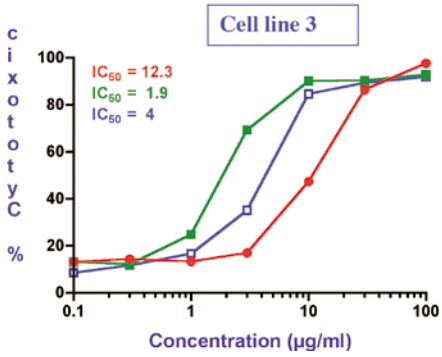


Figure 2:
Effect of different solvent extracts of the plant SN-001 on the proliferative index of multiple cancer cells

> "The Tecan platform allows simultaneous testing of hundreds of compounds against many assays relevant to cancers, inflammatory diseases and metabolic disorders"

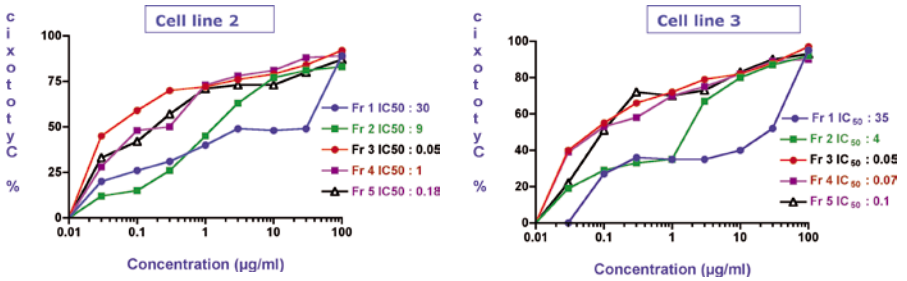


Figure 3:
Bioacitivity-guided fractionation using multiple solvent extracts of the plant SN-001 on the proliferative index of multiple cancer cells

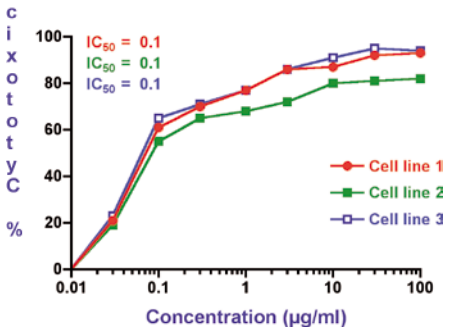


Figure 4:
Isolation of a pure molecule from the active fraction 3 with antiproliferative activity on cancer cells using bioacitivity-guided fractionation

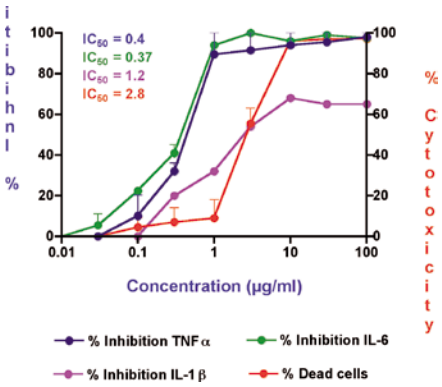


Figure 5:
Effect of a synthetic compound on the production of proinflammatory cytokines in LPS-stimulated monocytes

We have identified extracts with unique (single target) as well as dual activities (two or more targets). The activity of some of these potential hits is graphically represented in Figures 1–5; these data are indicative of natural product scaffolds within the extracts that may be responsible for dual or unique target effects. Automating our assays using the Freedom EVO 200 system has made life much easier and has significantly contributed to reducing the hit-to-lead transformation time.

The set-up was fully installed at the NPRC and running three different experimental protocols within a month of its arrival in Mumbai, thanks to the excellent training that Tecan provided to its Indian distributor, Bioscreen. We are planning to increase the output to 20,000 or more data points per week by switching to 384-well format assays, which should have happened by the end of 2006. Nicholas Piramal India Ltd is already beginning to see the benefits of the high throughput screening platform: in just two months, the automated screening facility has managed to complete a workload that would have taken an entire year to complete when performed manually.

Summary
High throughput screening on an automated platform is an efficient way for establishing robust assays that can yield reliable, reproducible data in phenotypic cell-based screens. Complex cellular assays can be standardized for screening natural product-based libraries, bioactivity-guided fractionation, isolation of active molecules and reducing hit-to-lead identification time. Our investment in the technologically advanced Freedom EVO 200 system has helped us increase sample source and assay throughput, speed up chemical isolation and rapidly identify common compounds by biological and chemical means.