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Accelerated approach of discovering plant-derived drug leads for treatment of tuberculosis

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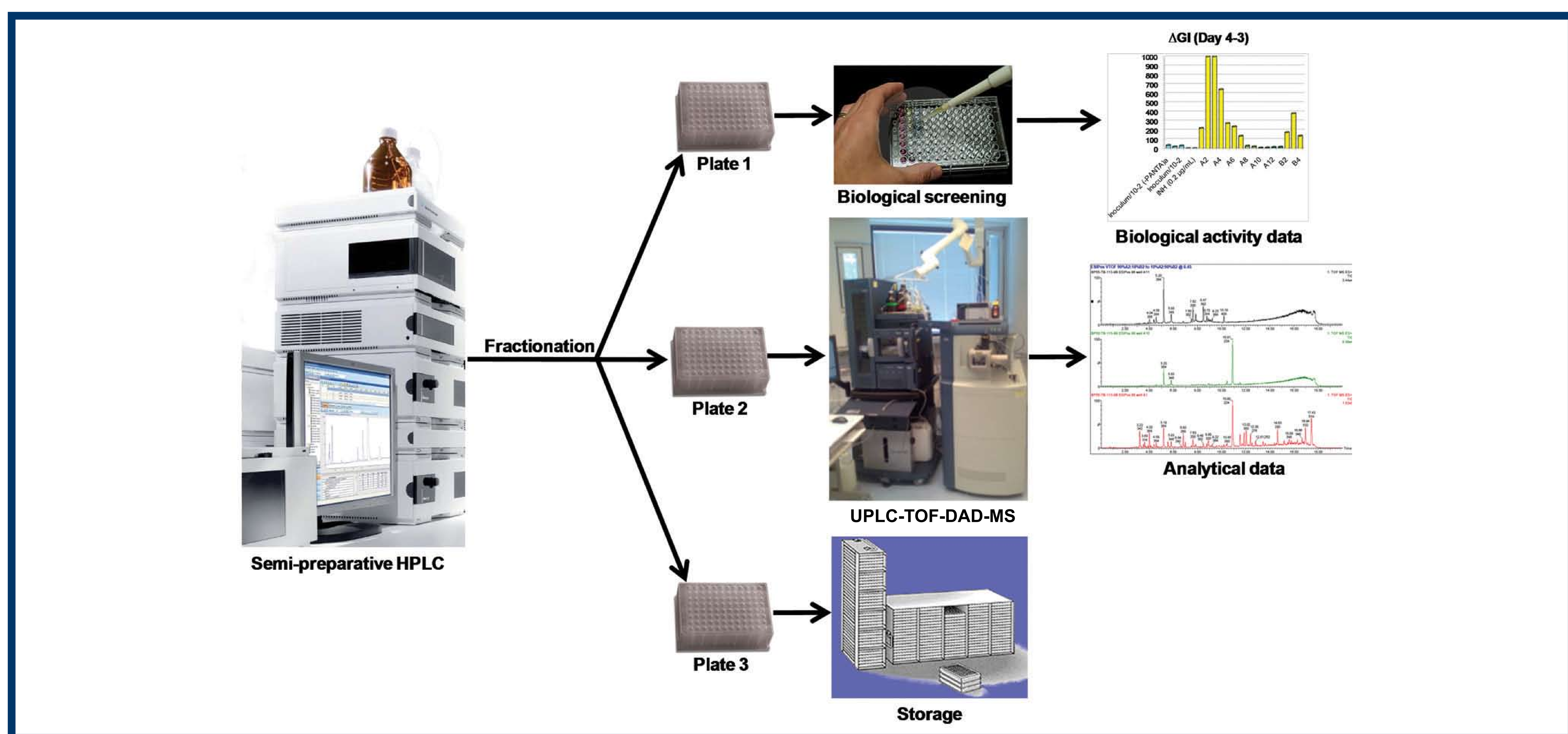

Figure 1: Accelerated drug discovery approach using 96 well plate fractionation

INTRODUCTION

The bioprospecting research group at CSIR biosciences undertakes research that taps into South Africa's plant biodiversity with the aim of discovering natural products that can be used to develop new medicines effective against tuberculosis (TB). To achieve this, the group has implemented the accelerated 96 well plate fractionation approach (**Figure 1**) to speed up the identification of active ingredients and interesting chemical scaffolds for subsequent medicinal chemistry developments, and to prevent the repeated discovery of known and undesired compounds, thereby saving time and resources.

To demonstrate the effectiveness of the accelerated approach, one of the organic plant extracts that displayed good anti-TB activity (MIC ≤ 250 µg/ml) was fractionated and screened for biological activity. The active fractions were analysed by UPLC-TOF-DAD-MS for UV, accurate mass and MS-MS fragmentation data to identify the compounds present. By using the data to search the Dictionary of natural products, scientists could identify the compound responsible for activity and could make upfront go/no go decisions, depending on the compound's desirability as a drug lead.

RESULTS AND DISCUSSION

The plant extract (TB-113-9B) was screened against the *Mycobacterium tuberculosis* H37Rv strain in the BACTEC assay with MIC = 125 µg/mL. Fractionation of the organic extract (TB-113-9B) yielded three sets of fractions. The first set was sent for analytical analysis, the second set was sent for biological screening and the third set was kept in storage. This approach speeds up the development time as correlation of chemical data to bioactive fractions can occur over a much shorter time frame. The fractions were screened at 50.00 µg/mL. According to the biological screening results, only fraction A10 had inhibitory activity against the drug sensitive strain. Other fractions were inactive.

The UPLC-TOF-DAD-MS data generated for the crude extract A1 and the fractions A10 and A11 are shown in **Figure 2**.

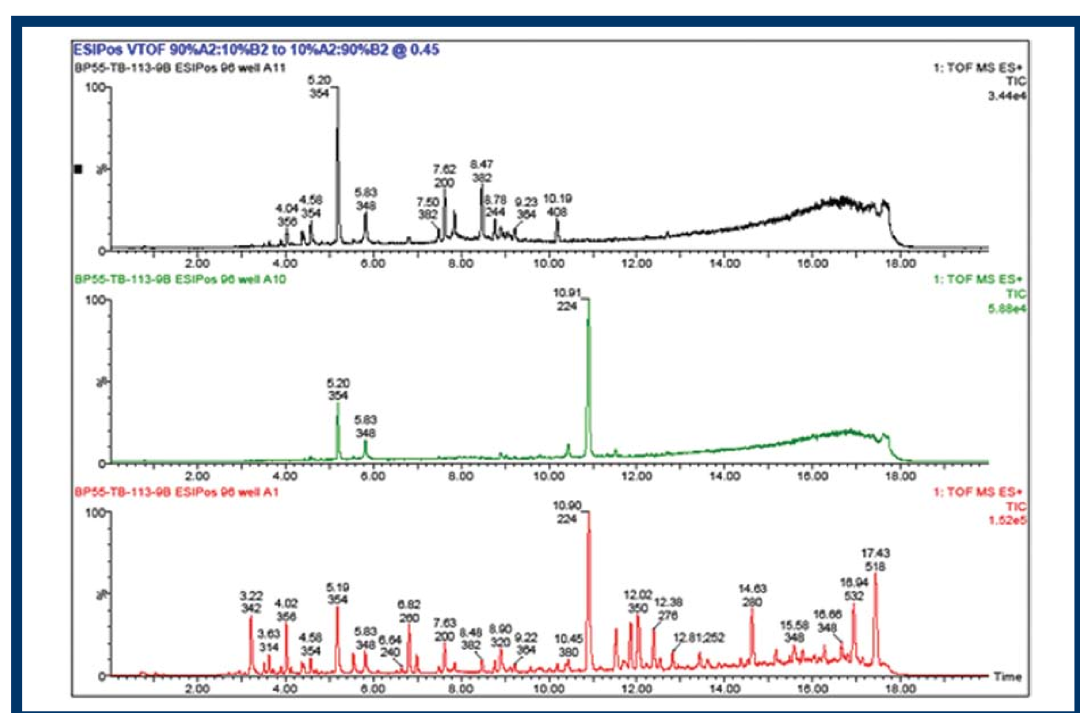

Figure 2: Total Ion Chromatograms (TIC) (positive modes) of extract A1 and fractions A10 and A11

The chromatogram of the active fraction **A10** shows peaks at retention times 10.9, 5.8 and 5.2. The peaks at 5.2 and 5.8 are present in the inactive fraction **A11** and the peak at 10.9 is absent suggesting that the peak at Rt 10.9 belongs to the active constituent.

The ESI TOF MS (positive mode) of peak at Rt 10.9 showed molecular ions with *m/z* 224.2002 [M+H]+ corresponding to a mass of 223.1923, and the UV spectrum showed UV maxima peak at 256 nm. By comparing the data with those reported in literature, the compound was identified as pellitorine **(1)**, previously isolated from *Zanthoxylum capense* (Steyn, 1998). A literature search showed this compound to have anti-TB activity with an MIC of 25 µg/ml (Rukachaisirikul, 2004). The compound responsible for anti-TB activity was confirmed to be pellitorine **(1)**.

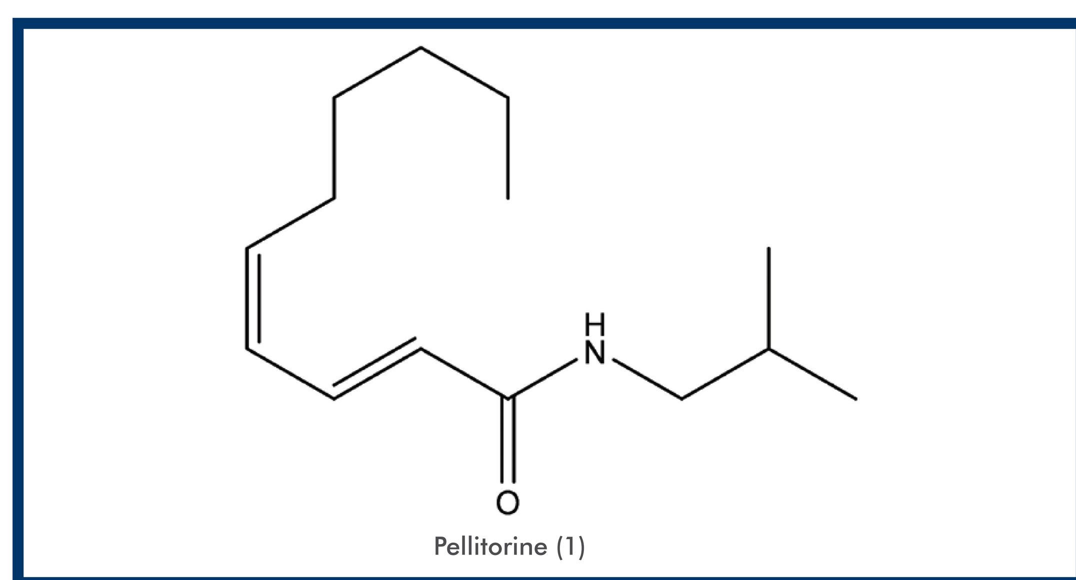

Figure 3: Structure of active compound

CONCLUSIONS

The approach to accelerate the identification of potentially new anti-TB leads has proved successful as de-replication has lead to the identification of a known compound. Pellitorine (**1**) identified from TB-113-9B has been previously reported to exhibit anti-TB activity. If the classical bioassay-guided fractionation approach was followed, the identification of the active compound would have taken a much longer time.

The compound will be subjected to structure activity relationship studies and computational modelling to identify potential sites for structural modification to improve efficacy and reduce cytotoxicity.

ACKNOWLEDGEMENTS

We acknowledge SANBI for the identification and collection of plant material. We thank Mr Nial Harding for advice on HPLC method development and Mr Eric Khorombi for preparation of the TB-113-9B plant extract. We acknowledge Dr Tracy Seaman and Dr Peter Smith from the University of Cape Town, Division of Pharmacology for biological screening of the plant extract against *M. aurum* and *M. tb.*, and Prof Namrita Lall and Ms Antoinette Labuschagne from the University of Pretoria for biological screening of samples against *M. tb*. Thanks to the CSIR Parliamentary Grant (PG) for funding the TB research and the NRF NEP/RISP for funding that allowed us to purchase the SYNAPT in collaboration with the University of Johannesburg.

CSIR researchers have successfully demonstrated the effectiveness of the accelerated approach to identify anti-TB constituents from plant extracts.

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