SL25 An *in vitro*: *in vivo* fusion system for optimised production of St. John's wort (*Hypericum perforatum* L.)

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The efficient production of St. John's wort (*Hypericum perforatum* L.) requires a fusion of growing systems in controlled environments to ensure that the biochemical profile of the resulting plant material has the highest possible quality. Wild harvested and cultivated St. John's wort has a broad diversity of chemotypes arising from spontaneous apomixis in seed development, pollenation, and environmental effects resulting in variable synthesis and accumulation of specific compounds. Therefore, an *in vitro* system for clonal propagation via cytokinin-induced *de novo* shoot organogenesis was developed to provide sterile, uniform plant material for investigations. Exposure of etiolated hypocotyls or sterile stem segments to a medium containing 5 µM thidiazuron (TDZ: N-phenyl-N'-(1,2,3-thidiazol-yl)-urea) for 6-9 days with subsequent transfer to a medium devoid of growth regulators resulted in the development of 25-40 shoots per explant. The regeneration protocol was used to generate a series of selected lines originating from a single seed. *In vitro* propagated plantlets of line SJW17 were transferred to a controlled environment greenhouse, acclimatized to a hydroponic system and grown to maturity for tissue collection. Flowers were harvested from 2-month-old plants and subjected to biochemical analyses. Hypericin, hyperforin and pseudohypericin were present in the flowers at comparable concentrations to previous reports for field-produced plant materials. Melatonin and serotonin, indoleamine neurohormones associated with circadian rhythms and anti-oxidation pathways, were quantified in the *in vitro* plantlets and the *in vivo* flower tissues. Melatonin was quantified in leaf (1.8 µg/g), flower (4.4 µg/g), stem (1.9 µg/g), and etiolated hypocotyls (59.8 µg/g). Radiolabel from ^{14}C-tryptophan was recovered as ^{14}C-melatonin in sterile plantlets indicating endogenous synthesis of the compound in St. John's wort. Analysis of the flower buds at six different stages revealed that serotonin was present during the tetrad stage of anther development while melatonin was detected at high levels during uninucleate mircospore development. Together, these investigations have demonstrated that a fusion of *in vitro* and *in vivo* systems can be effectively used for efficient production and discovery of novel compounds in St. John's wort and other medicinal plants.

SL26 Synthesis, cytotoxicity and antiplasmodial activity of new *indoloquinoline* derivatives

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Based on the original lead neocryptolepine or 5-methyl-5*H*-indolo[2,3-*b*]quinoline, an alkaloid from *Cryptolepis sanguinolenta*, a series of derivatives was prepared using a biradical cyclisation methodology. Starting from easily accessible products, this approach allowed the synthesis of hitherto unknown compounds with a varied substitution pattern. As a result of steric hindrance, preferential formation of the 3-substituted isomers over the 1-substituted isomers was observed when cyclising *N*(3-substituted-phenyl)-*N*'[2-(2-trimethylsilylethynyl)phenyl]carbodiimides.

All compounds were evaluated for their activity against chloroquine-sensitive as well as chloroquine-resistant *Plasmodium falciparum* strains, and for their cytotoxicity on human MRC-5 cells. Mechanisms of action were investigated by testing inhibition of β-haematin formation, and DNA interactions (DNA-methylgreen assay).

Neocryptolepine derivatives with a higher antiplasmodial activity and a lower cytotoxicity than the original lead have been obtained. This selective antiplasmodial activity was associated with inhibition of β-haematin formation. 2-Bromoneocryptolepine was the most selective compound with an IC_{50} value against chloroquine-resistant *P. falciparum* of 4.0 µM in the absence of cytotoxicity (IC_{50} > 32 µM). Although cryptolepine, a known lead for antimalarials also originally isolated from *Cryptolepis sanguinolenta*, was more active (IC_{50} 2.0 µM), 2-bromoneocryptolepine showed a low affinity for DNA, in contrast to cryptolepine.

Although some neocryptolepine derivatives with a higher antiplasmodial activity than 2-bromoneocryptolepine were obtained, these compounds also showed a higher affinity for DNA and/or a more pronounced cytotoxicity. Therefore, 2-bromo-neocryptolepine is considered as the most promising lead from the present work for new antimalarial agents.