Isolation of modulators of Organic Anion Transporting Proteins (OATPs) from *Rollinia emarginata* Schlecht. (Annonaceae)

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INTRODUCTION

Organic Anion Transporting Polypeptides (OATPs) comprise a superfamily of sodium-independent membrane transporters that are involved in transporting numerous endogenous and exogenous substances. For instance, these transporters are responsible for the uptake of important drugs including the anticancer drugs methrotexate, SN-38, paclitaxel, and docetaxel. Eleven human OATPs have been described to date and are expressed in different tissues such as intestine, liver, kidney, and brain. OATP1B1 and OATP1B3 are expressed specifically in liver and both have a broad substrate specificity, transporting compounds like bile salts, hormones, conjugated hormones, eicosanoids, cyclic and linear peptides, toxins, and numerous drugs. Despite the overlapping substrate specificity between OATP1B1 and OATP1B3, specific substrates for each transporter are known.[1]

Recently, reports have shown altered expression of OATPs in cancer cells, specifically breast and colon cancer.[2-3] Lee et al.[2] were able to show that overexpression of OATP1B3 conferred apoptotic resistance in colon cancer. Therefore, understanding the role of OATPs in anticancer drugs uptake into tumor cells may contribute to the identification of mechanisms of chemotherapy resistance.

Natural products have been identified as potential modulators of OATPs. Several herbal extracts were reported to have inhibitory effects on OATP2B1 mediated uptake of estrone-3-sulfate.[4] In addition, flavonoids have been suggested as a novel class of OATP1B1 modulators.[5] However, no systematic natural-products-based screening has been reported for the identification of novel OATP modulators.

The long-term goal of our study is to discover OATP modulators that may further elucidate the transport mechanism of these complex proteins and ultimately lead to a novel therapeutic approach to cancer treatment. To search for novel modulators of OATP1B1 and OATP1B3, over 200 plant extracts were tested. Based on primary screening results, *Rollinia emarginata* was selected for further exploration using a bioassay-guided fractionation approach..

EXPERIMENTAL SECTION

Plant material. Above-ground biomass of *Rollinia emarginata* was collected in Argentina in February 1999.

Extraction, isolation and identification. Plant material (562 g) was extracted four times with MeOH:DCM 1:1 for 16 hours. The combined extracts were evaporated to dryness *in vacuo* at 35°C. The residue (37.7 g) was suspended in MeOH 90% and successively partitioned with different solvents (figure 1). Several chromatographic techniques were applied to separate the subfractions depending on polarity and quantity. Isolated compounds were chemically elucidated using 1D and 2D NMR, IR, UV, LCMS, and HRMS experiments.

NMR experiments. NMR experiments were performed in a Bruker AVIII 500 instrument with a dual C/H cryoprobe. Standard ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC and HMBC were recorded of each of the isolated pure compounds.

LCMS. LC Agilent 1200 system with a 6300 Series Ion Trap detector was used for LCMS experiments. An Agilent Eclipse XDB C18 5µm (4.6×150mm) column was used and different gradients of acetonitrile:water were applied as a mobile phase depending on the polarity of samples.

HRMS. HRMS was obtained with a LCT Premier Waters Corp.

OATPs uptake experiments. Plant extract, fractions and pure compounds (0.3 µg/mL) were tested for effects on OATP1B1- and OATP1B3-mediated uptake of ^{3}H-estradiol-17β-glucuronide (0.1mM) and ^{3}H-estrone-3-sulfate (1mM) for 5 minutes at 37°C.[6]

RESULTS

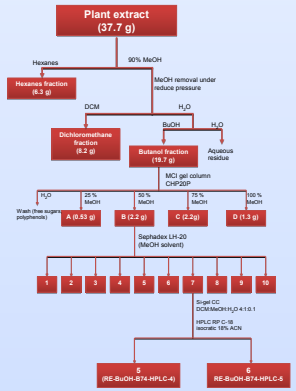

FIGURE 1 Separation diagram of *Rollinia emarginata* organic extract.

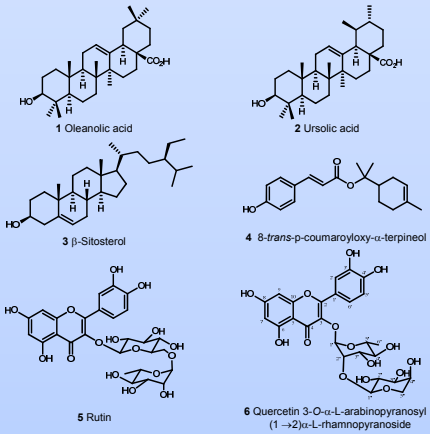

FIGURE 2 Chemical structure of OATP modulators isolated from *Rollinia emarginata* hexanes fractions (**1**-**4**) and butanol fractions (**5** and **6**)

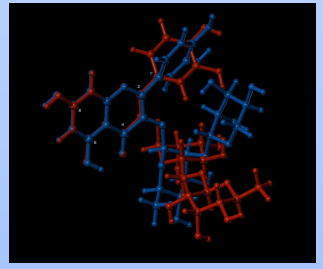

FIGURE 3 Minimum energy conformation of **5** (red) and **6** (blue). Minimization was performed in SYBYL with MM2 and alignment-by-atom protocols.

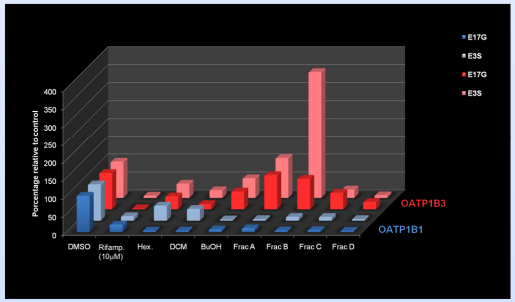

FIGURE 4 Inhibition of OATP1B1 and OATP1B3-mediated estradiol-17β-glucuronide (E17G) and estrone-3-sulfate (E3S) uptake as percentage of vehicle control by fractions. Positive control is 10 µM rifampicin.

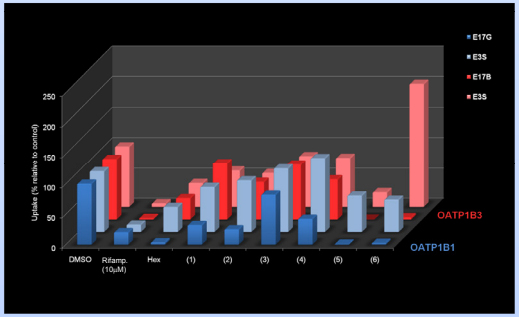

FIGURE 5 Inhibition of OATP1B1 and OATP1B3-mediated estradiol-17β-glucuronide (E17B) and estrone-3-sulfate (E3S) uptake as percentage of vehicle control by isolated compounds **1**-**6**. Positive control is 10 µM rifampicin.

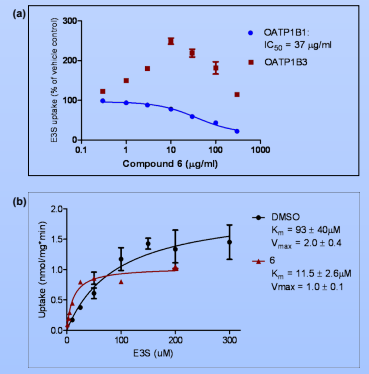

FIGURE 6 **(a)** Effect of compound **6** in OATP1B1- and OATP1B3-mediated estrone-3-sulfate uptake. **(b)** Effect of 50 mM of compound **6** in the kinetics of OATP1B3-mediated estrone-3-sulfate (E3S) uptake.

CONCLUSIONS

Novel OATP1B1 and OATP1B3 modulators were successfully isolated from *Rollinia emarginata* organic extract using a bioassay guided approach.

Quercetin 3-*O*-α-L-arabinopyranosyl (1→2)α-L-rhamnopyranoside (**6**) was characterized as an inhibitor of both OATP1B1- and OATP1B3-mediated uptake of estradiol-17β-glucuronide. This compound however, stimulates OATP1B3-mediated uptake of estrone-3-sulfate by increasing the apparent affinity of the substrate for the transporter.

FUTURE WORK

Investigate the relationship between modulation of OATP1B1 and OATP1B3 and structure of flavonoids, glycoflavonoids, and related phenolic compounds.

Continue the screening of plant extracts and identification of novel and structurally diverse modulators of OATP1B1 and OATP1B3

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