HBOI-381

Nicellin A

C_{26}H_{36}O

286.45

Nicella sp.

- Isolation scheme (Visio) attached
- Hplc method (trace attached)

Method: Vydac C18 Protein and Peptide, 4.6 x 250mm, 10μ flow 1 ml/min, detection: PDA: UV (extracted at 220 nm, black); ELSD (green dotted)
A: H_{2}O:CH_{3}CN (95:5, v/v), B: CH_{3}CN
t=0 min A:B (90:10, v/v), t=20 min (100%B), t=28 min (100%B)

LC-MS method

Method: Vydac C18 Protein and Peptide, 2.1x150 mm, flow 0.2 ml/min
A: H_{2}O (0.1% formic acid), B:CH_{3}CN (0.1% formic acid)
t=0 min A:B (90:10, v/v), t=15 min (100%B), t=21 min (100%B), t=22.1 min A:B(90:10, v/v) using a linear gradient

- ^1H (600 MHz)  
- CDCl_{3}  
- CD_{3}OD (Methanol-d_{4})  
- CDCl_{3}/CD_{3}OD

- ^13C (150 MHz)  
- CDCl_{3}  
- CD_{3}OD (Methanol-d_{4})  
- CDCl_{3}/CD_{3}OD

- solubility  
- CHCl_{3}/MeOH (9:1)  
- MeOH  
- DMSO

- Estimated purity >90%
- Sample weight _19.5_mg

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Date: 1/28/13
NICELLA SPECIES
100 g of sample extracted / 56 g of sample remaining
Inner "bones" crushed & cut into manageable pieces for blender.
Ground & extracted 2x w/250ml EtOAc; EtOH 9:1,
2x w/250ml EtOH, 2x w/250ml MeOH
GARU 1-3-1 2.915 g

2.9 g portioned 3x w/EtOAc: H₂O

GARU 1-3-2
EtOAc partition
0.7632 g

~0.5942 g run on 12 g NP Rf Gold column, CV 16.8 ml, 30 ml/min
CombiFlash Companion 50 psi system
Heptane to DCM gradient; Wash 1: DCM to MeOH 75:25
Wash 2: DCM to MeOH 1:1; Wash 3: MeOH

GARU 1-3-
Weight
Tubes
6
377.2 mg
9-25

A portion of this fraction was brought to 73 mg/ml
solution in MeOH for further purification via Waters Prep
LC-MS system. Luna C18, 19 X 350mm column.

Flow: 23.5 ml/min
A: H₂O:ACN 95:5
B: ACN 100%
100% A for 0.5 min, 100% B by 8 min, hold at
100% B to 12 min, collection stops at 12 min,
tube size 16/150 mm
Five runs

Day 1 runs

Day 2 runs

Samples Sam30-8205AW
and Sam30-8305AW
were combined to give Sam30-8401AW
HPLC/ELSD, LC-MS, proton and Carbon NMR
were performed on this sample
D-2000 Elite HPLC System Manager Report

Sample Name: HBOI-381
Sample Description: HBOI-381

Retention Time (min)

Absorbance (AU)

Wavelength (nm)

Ch 1 — Ch 2
Acquisition Method: autosamp_30mn_UV220_wELSD
Column Type: Vydac C18
Pump A Solvent A: H2O/5% ACN
Method Description:

Pump A Solvent B: ACN