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# Online Study on Metabolites Profiles of Sixteen Clausenamide Enantiomers in vitro by Liquid Chromatography/Quadrupole Ion Trap/Time-of-Flight Mass Spectrometry

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## 1. Introduction

Clausenamide is a chiral nootropic drug extracted from *Clausena lansium* (Lourd.) Skeels. It has been synthesized in the Institute of Materia Medica and has been studied in the clinical trials of Phase II. The nootropic effect of (-)-clausenamide is much better than that of (+)-clausenamide and the latter has stronger toxicity than the former. In addition, including the (-)-clausenamide and (+)-clausenamide, all clausenamide enantiomers have different effects on colony form frequency in human embryonic neural stem cells, and as well as on induction of LTP in hippocampal of rat, and on concentration of nerve cell cytosolic free calcium.

The biotransformations of sixteen clausenamide enantiomers have been investigated *in vitro* in this study. The stereo-selectivity on metabolism and differences of sixteen clausenamide enantiomers has been elucidated by means of identification of metabolites and explanation of pathways.

## 2. Method and Materials

### Sample Preparation

(1) Phenobarbital sodium was administrated via i.p. to S.D. rats. Administration dose was 60 mg/kg, once per day for 3 days.
(2) Liver was removed to produce the liver microsome. BCA method was used to confirm the protein concentration.
(3) G-6-p DH, microsome, G-6-p, NADP, NADH, as well as $MgCl_2$ were dissolved with Tris-KCl buffer.
(4) Each substance with the optimized concentration was skipped into tubes.
(5) Pre-incubate the system without clausenamide at 37°C, 2 min.
(6) Turn the system on with spiked clausenamide and the other 15 isomers.
(7) Incubate the system about 2 h.
(8) Precipitate protein with isopyknic acetonitrile to terminate the reaction.
(9) Centrifuge the mixture at 10000 rpm, 15 min.
(10) Extract the supernatantliquor with isopyknic ethyl acetate twice.
(11) Blow-dry the ethyl acetate with N2 flow at 40°C.
(12) Dissolve the leaving with 500 µL acetonitrile.
(13) Filter the solution with 0.45 µm filter.

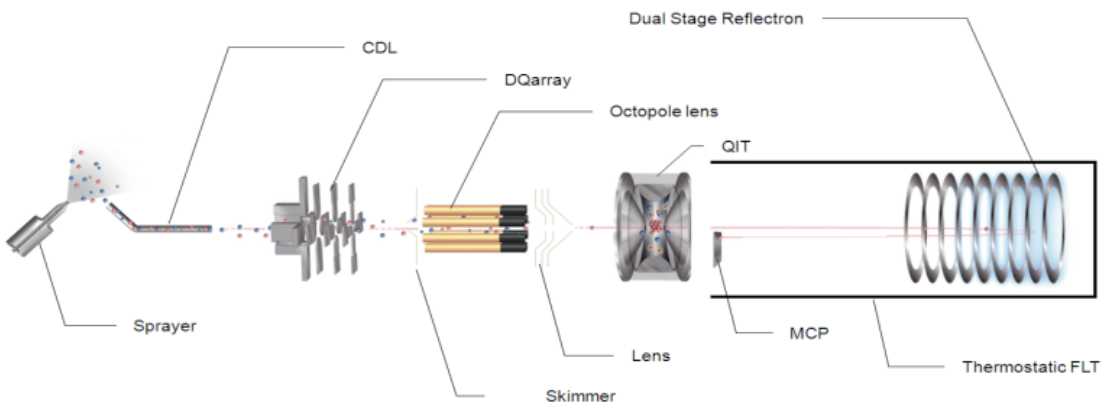


Fig. 1 Schematic diagram of the LCMS-IT-TOF

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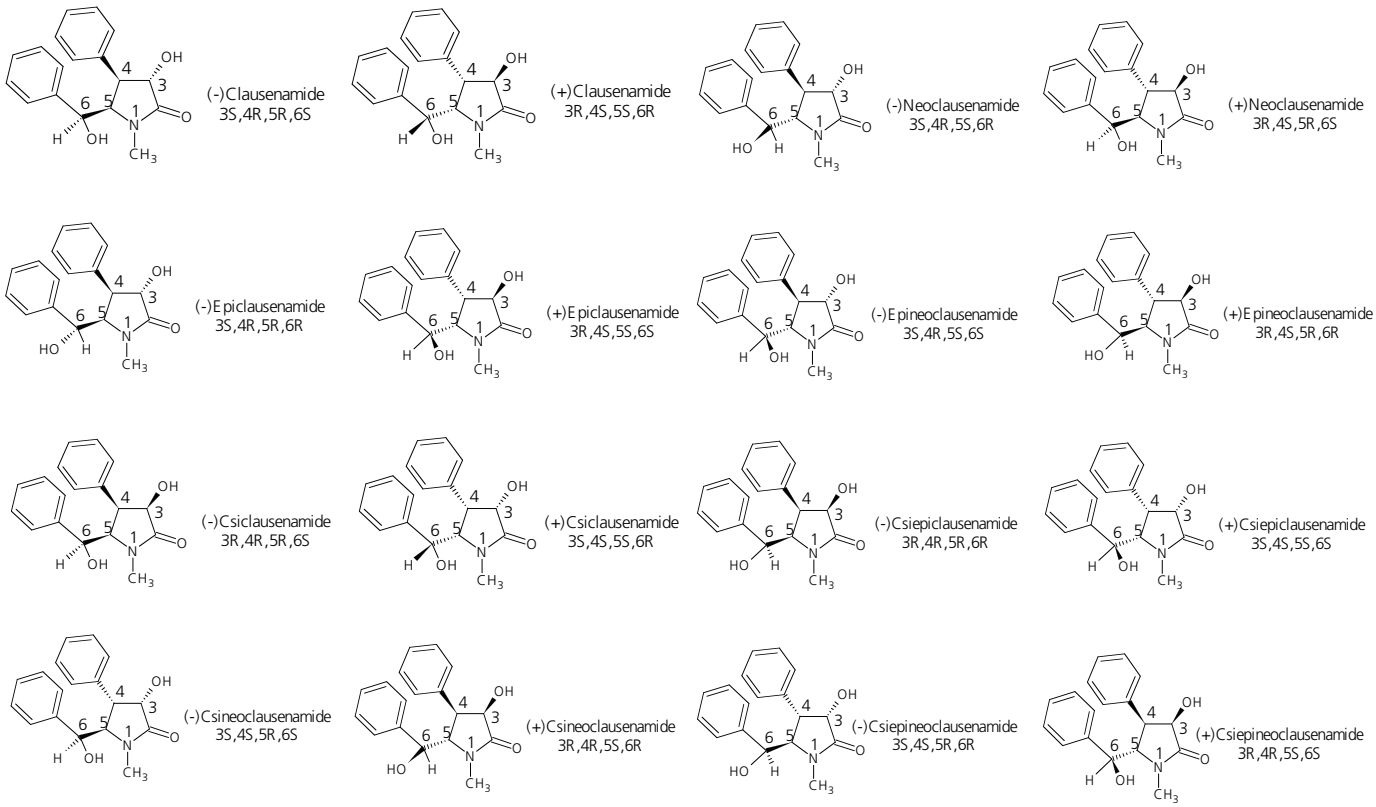


Fig. 2 Structure of the (-)-clausenamide and the other Isomers

**Analytical conditions**

UHPLC (Prominence UFLCXR system)

The analyses were performed on a Shimadzu Prominence UFLCXR system equipped with LC-20ADXR pumps, a CTO-20AC column oven, an SIL-20ACXR autosampler, a DGU-20A5 degasser and a CBM-20A communication bus module.

| | |
|---|---|
| Mobile phase A | : 0.1% ammonia |
| Mobile phase B | : methanol |
| Column | : ODS, 250 mmL. × 4.6 mmi.d., 5 µm |
| Flow rate | : 0.8 mL/min (split, MS/waste = 0.3/0.5) |
| Wavelength | : 210 nm |
| Column oven | : 40°C |
| Injection volume | : 10 µL |

MS (LCMS-IT-TOF)

| | |
|---|---|
| Ionization | : ESI+ |
| Ionization voltage | : + 4.5 kV |
| Scan Range | : *m/z* 50-1,000 |
| Nebulizing gas | : nitrogen, 1.5 L/min |
| Drying gas | : nitrogen, 10 L/min |
| Collision gas | : argon |
| CDL temperature | : 200°C |
| Heat block temperature | : 250°C |
| Detector voltage | : 1.70 kV |

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## 3. Results and Discussion.

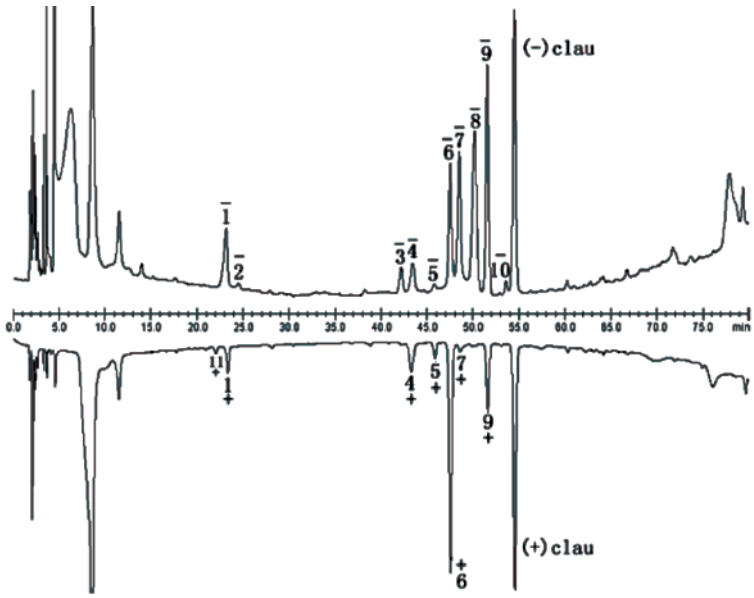


Fig. 3 (+)(-)-clausenamide NADPH-generating system HPLC-UV mirror chromatograms

Table 1 clausenamide enantiomers and their metabolites

| Groups | Compounds | Formula | Groups | Compounds | Formula | Groups | Compounds | Formula | Groups | Compounds | Formula |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group-1 | (−)clau | $C_{18}H_{19}NO_3$ | Group-4 | (+)neoclau-M11 | $C_{17}H_{17}NO_4$ | Group-9 | (−)csiclau-M8 | $C_{17}H_{17}NO_3$ | Grooup-13 | (−)csineoclau | $C_{18}H_{19}NO_3$ |
| | (−)clau-M1 | $C_{18}H_{19}NO_4$ | Group-5 | (−)epiclau | $C_{18}H_{19}NO_3$ | | (−)csiclau-M10 | $C_{17}H_{15}NO_3$ | | (−)csineoclau-M2 | $C_{18}H_{19}NO_4$ |
| | (−)clau-M3 | $C_{17}H_{17}NO_4$ | | (−)epiclau-M3 | $C_{18}H_{19}NO_4$ | | (−)csiclau-M11 | $C_{18}H_{19}NO_4$ | | (−)csineoclau-M4 | $C_{18}H_{17}NO_4$ |
| | (−)clau-M6 | $C_{18}H_{19}NO_4$ | | (−)epiclau-M4 | $C_{18}H_{19}NO_4$ | | (−)csiclau-M13 | $C_{18}H_{19}NO_3$ | | (−)csineoclau-M6 | $C_{18}H_{17}NO_3$ |
| | (−)clau-M7 | $C_{17}H_{17}NO_3$ | | (−)epiclau-M6 | $C_{18}H_{17}NO_3$ | | (−)csiclau-M14 | $C_{18}H_{17}NO_3$ | | (−)csineoclau-M7 | $C_{17}H_{15}NO_3$ |
| | (−)clau-M8 | $C_{18}H_{19}NO_4$ | | (−)epiclau-M7 | $C_{17}H_{17}NO_3$ | | (−)csiclau-M15# | $C_{17}H_{17}NO_4$ | | (−)csineoclau-M8 | $C_{18}H_{17}NO_3$ |
| | (−)clau-M9 | $C_{18}H_{17}NO_3$ | | (−)epiclau-M8 | $C_{18}H_{19}NO_4$ | Group-10 | (+)csiclau | $C_{18}H_{19}NO_3$ | Group-14 | (+)csineoclau | $C_{18}H_{19}NO_3$ |
| | (−)clau-M10 | $C_{17}H_{15}NO_3$ | | (−)epiclau-M9 | $C_{18}H_{19}NO_4$ | | (+)csiclau-M5 | $C_{18}H_{19}NO_4$ | | (+)csineoclau-M2 | $C_{18}H_{19}NO_4$ |
| Group-2 | (+)clau | $C_{18}H_{19}NO_3$ | Group-6 | (+)epiclau | $C_{18}H_{19}NO_3$ | | (+)csiclau-M6 | $C_{17}H_{17}NO_4$ | | (+)csineoclau-M3 | $C_{18}H_{19}NO_4$ |
| | (+)clau-M1 | $C_{18}H_{19}NO_4$ | | (+)epiclau-M3 | $C_{18}H_{19}NO_4$ | | (+)csiclau-M7 | $C_{17}H_{17}NO_3$ | | (+)csineoclau-M4 | $C_{18}H_{17}NO_4$ |
| | (+)clau-M3# | $C_{17}H_{17}NO_4$ | | (+)epiclau-M7 | $C_{17}H_{17}NO_3$ | | (+)csiclau-M10# | $C_{17}H_{15}NO_3$ | | (+)csineoclau-M6 | $C_{18}H_{17}NO_3$ |
| | (+)clau-M4 | $C_{17}H_{17}NO_4$ | | (+)epiclau-M9 | $C_{18}H_{19}NO_4$ | | (+)csiclau-M11 | $C_{18}H_{19}NO_4$ | | (+)csineoclau-M7 | $C_{17}H_{15}NO_3$ |
| | (+)clau-M5 | $C_{18}H_{19}NO_5$ | | (+)epiclau-M10# | $C_{17}H_{15}NO_3$ | | (+)csiclau-M13 | $C_{18}H_{19}NO_3$ | | (+)csineoclau-M8 | $C_{18}H_{17}NO_3$ |
| | (+)clau-M6 | $C_{18}H_{19}NO_4$ | | (+)epiclau-M11# | $C_{18}H_{17}NO_4$ | | (+)csiclau-M14 | $C_{18}H_{17}NO_3$ | | (+)csineoclau-M10 | $C_{17}H_{17}NO_3$ |
| | (+)clau-M7 | $C_{17}H_{17}NO_3$ | | (+)epiclau-M12# | $C_{18}H_{17}NO_3$ | | (+)csiclau-M16 | $C_{18}H_{19}NO_4$ | | (+)csineoclau-M11 | $C_{18}H_{19}NO_3$ |
| | (+)clau-M9 | $C_{18}H_{17}NO_3$ | Group-7 | (−)epineoclau | $C_{18}H_{19}NO_3$ | Group-11 | (−)csiepiclau | $C_{18}H_{19}NO_3$ | Group15 | (−)csiepineoclau | $C_{18}H_{19}NO_3$ |
| | (+)clau-M11 | $C_{18}H_{19}NO_5$ | | (−)epineoclau-M2 | $C_{18}H_{19}NO_4$ | | (−)csiepiclau-M2 | $C_{18}H_{19}NO_4$ | | (−)csiepineoclau-M2 | $C_{18}H_{19}NO_4$ |
| | (+)clau-M12# | $C_{18}H_{17}NO_4$ | | (−)epineoclau-M3 | $C_{18}H_{19}NO_4$ | | (−)csiepiclau-M4 | $C_{17}H_{17}NO_4$ | | (−)csiepineoclau-M3 | $C_{18}H_{19}NO_4$ |
| Group-3 | (−)neoclau | $C_{18}H_{19}NO_3$ | | (−)epineoclau-M4 | $C_{18}H_{17}NO_4$ | | (−)csiepiclau-M5 | $C_{18}H_{19}NO_3$ | | (−)csiepineoclau-M4 | $C_{18}H_{17}NO_4$ |
| | (−)neoclau-M3 | $C_{18}H_{19}NO_4$ | | (−)epineoclau-M7 | $C_{17}H_{15}NO_3$ | | (−)csiepiclau-M7 | $C_{18}H_{19}NO_4$ | | (−)csiepineoclau-M6 | $C_{18}H_{17}NO_3$ |
| | (−)neoclau-M4 | $C_{18}H_{19}NO_4$ | | (−)epineoclau-M8 | $C_{18}H_{17}NO_3$ | | (−)csiepiclau-M8 | $C_{17}H_{15}NO_3$ | | (−)csiepineoclau-M7 | $C_{17}H_{15}NO_3$ |
| | (−)neoclau-M5 | $C_{18}H_{17}NO_4$ | | (−)epineoclau-M9 | $C_{18}H_{17}NO_3$ | | (−)csiepiclau-M9 | $C_{18}H_{17}NO_3$ | | (−)csiepineoclau-M8 | $C_{18}H_{17}NO_3$ |
| | (−)neoclau-M7 | $C_{17}H_{17}NO_3$ | Group-8 | (+)epineoclau | $C_{18}H_{19}NO_3$ | | (−)csiepiclau-M10 | $C_{17}H_{17}NO_3$ | | (−)csiepineoclau-M11# | $C_{17}H_{17}NO_3$ |
| | (−)neoclau-M8 | $C_{17}H_{15}NO_3$ | | (+)epineoclau-M2 | $C_{18}H_{19}NO_4$ | Group-12 | (+)csiepiclau | $C_{18}H_{19}NO_3$ | Group-16 | (+)csiepineoclau | $C_{18}H_{19}NO_3$ |
| | (−)neoclau-M9 | $C_{18}H_{19}NO_4$ | | (+)epineoclau-M7 | $C_{17}H_{15}NO_3$ | | (+)csiepiclau-M2 | $C_{18}H_{19}NO_4$ | | (+)csiepineoclau-M2 | $C_{18}H_{19}NO_4$ |
| | (−)neoclau-M10 | $C_{18}H_{17}NO_3$ | | (+)epineoclau-M8 | $C_{18}H_{17}NO_3$ | | (+)csiepiclau-M7 | $C_{18}H_{19}NO_4$ | | (+)csiepineoclau-M3 | $C_{18}H_{19}NO_4$ |
| Group-4 | (+)neoclau | $C_{18}H_{19}NO_3$ | | (+)epineoclau-M9 | $C_{18}H_{17}NO_3$ | | (+)csiepiclau-M10 | $C_{17}H_{17}NO_3$ | | (+)csiepineoclau-M4 | $C_{18}H_{17}NO_4$ |
| | (+)neoclau-M4 | $C_{18}H_{19}NO_4$ | Group-9 | (−)csiclau | $C_{18}H_{19}NO_3$ | | (+)csiepiclau-M11 | $C_{18}H_{19}NO_4$ | | (+)csiepineoclau-M6 | $C_{18}H_{17}NO_3$ |
| | (+)neoclau-M7 | $C_{17}H_{17}NO_3$ | | (−)csiclau-M1 | $C_{18}H_{19}NO_4$ | | (+)csiepiclau-M12 | $C_{18}H_{19}NO_5$ | | (+)csiepineoclau-M7 | $C_{17}H_{15}NO_3$ |
| | (+)neoclau-M8 | $C_{17}H_{15}NO_3$ | | (−)csiclau-M5 | $C_{18}H_{19}NO_4$ | | (+)csiepiclau-M14 | $C_{18}H_{19}NO_5$ | | (+)csiepineoclau-M8 | $C_{18}H_{17}NO_3$ |
| | (+)neoclau-M9 | $C_{18}H_{19}NO_4$ | | (−)csiclau-M6 | $C_{17}H_{17}NO_4$ | | (+)csiepiclau-M15 | $C_{17}H_{17}NO_4$ | | (+)csiepineoclau-M11 | $C_{17}H_{17}NO_3$ |
| | (+)neoclau-M10 | $C_{18}H_{17}NO_3$ | | (−)csiclau-M7 | $C_{17}H_{17}NO_3$ | | (+)csiepiclau-M16 | $C_{18}H_{17}NO_4$ | | (+)csiepineoclau-M12# | $C_{18}H_{19}NO_4$ |

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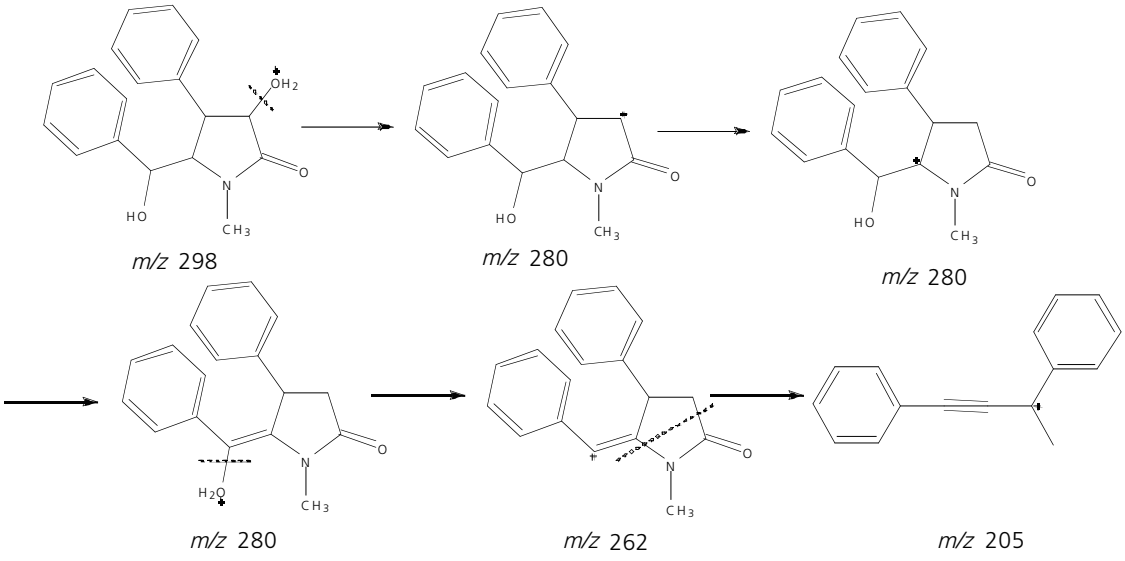


Fig. 4 Fragmentation pathway of clausenamide

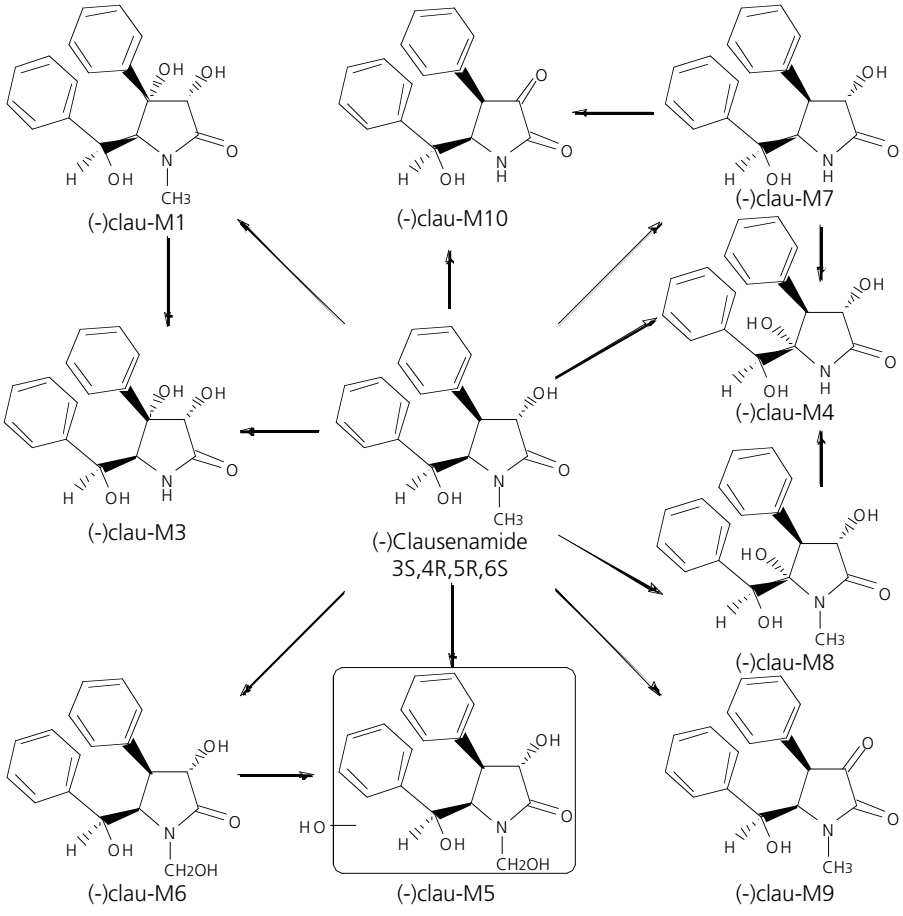


Fig. 5 (-)-clausenamide metabolic pathway

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## 4. Conclusions

The online system developed here can be applied for accurate identification of multicomponent in clausenamide enantiomers incubation system.
After the sixteen clausenamide enantiomers were incubated in the same system and at the same concentration respectively, different enantiomer plots different UV chromatograms and mass chromatograms.

More than 150 metabolites of the enantiomers have been detected by UV detection and ca. 120 metabolites by QIT/TOFMS from microsomal incubate system.
Considering the data which have already published and base on the chromatographic, UV and MS information, as well as chiral center factor, 114 metabolites have not been reported before.

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