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Stability study, method for the quantitation of a new antifilovirus agent in biological fluids and a preliminary study of its pharmacokinetics

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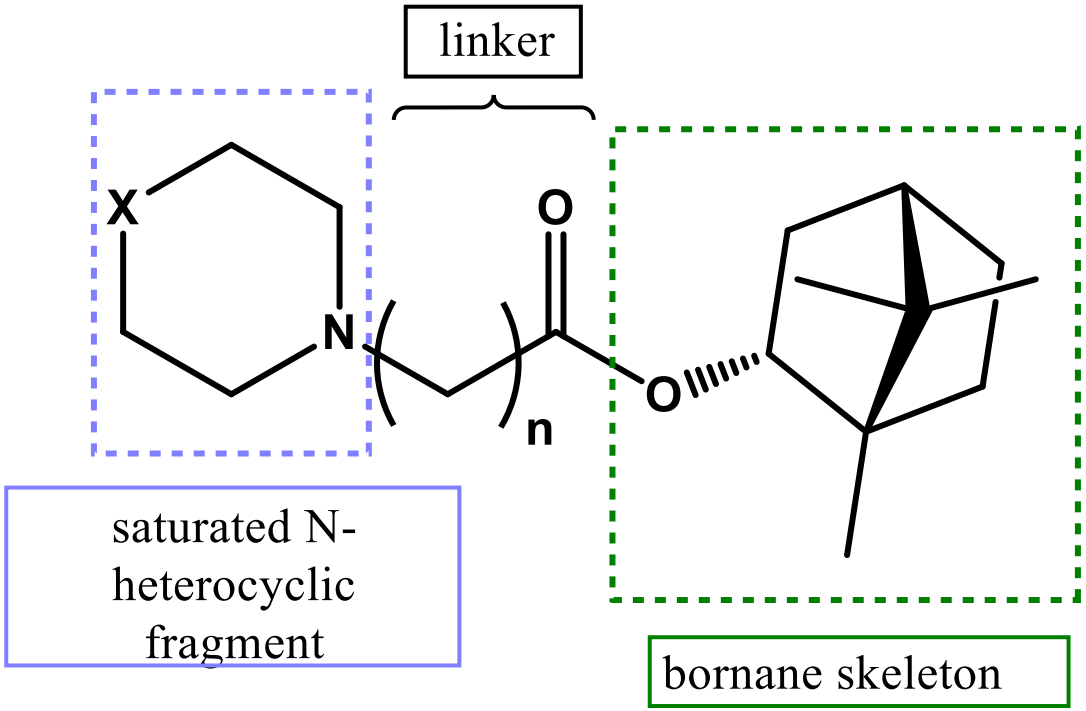
Stability study, method for the quantitation of a new antifilovirus agent in biological fluids and a preliminary study of its pharmacokinetics



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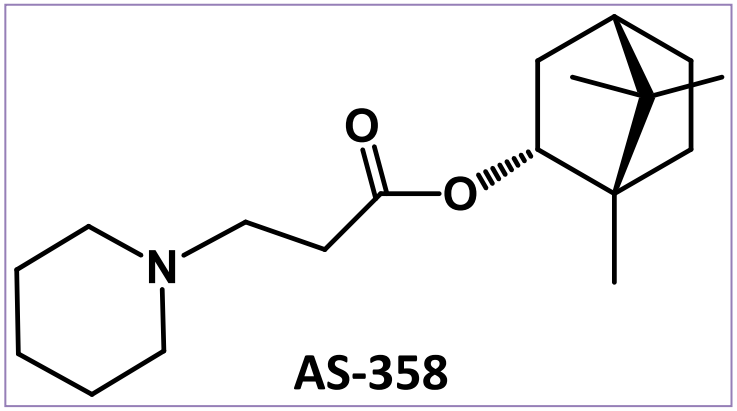
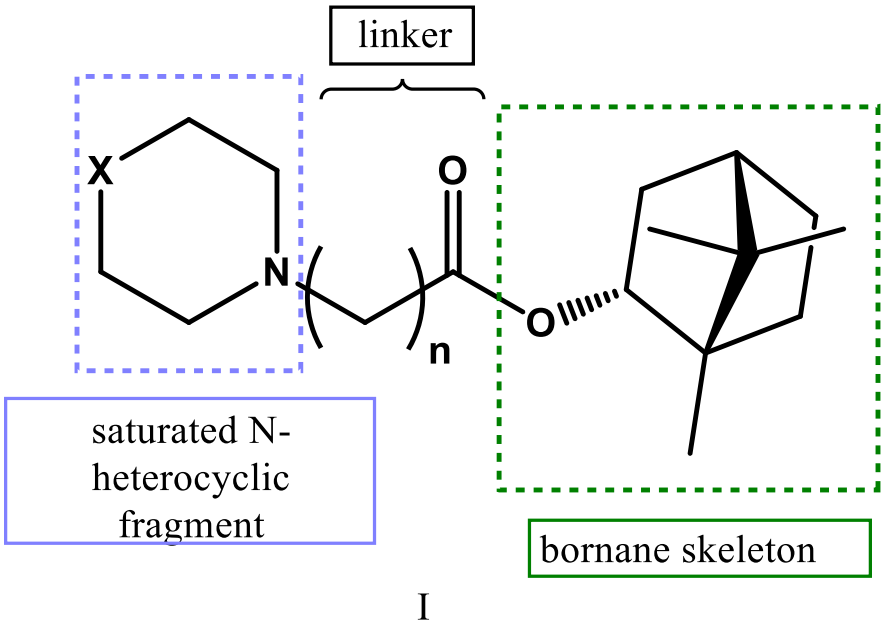


Abstract:
The stability of the new antifiloviral agent AS-358 consisting of borneol and 3-(piperidin-1-yl)propanoic acid, was studied in the blood and blood plasma of rats in vitro. It was found that in both matrices stabilized by EDTA or heparin, the compound is rapidly hydrolyzed at the ester bond. When sodium fluoride was added, the decomposition of the compound was significantly slowed down. This made it possible to develop and validate a method for the quantitative determination of the agent in whole rat blood. Analysis was performed by HPLC-MS/MS method using reverse phase chromatography. The developed method was used for a preliminary study of the pharmacokinetics of the agent AS-358 after its oral administration to rats, and it was shown that the concentration of AS-358 in the blood of animals reached 550 ng/ml after 1 hour, despite its instability in blood. Analogues of the agent which contain ether linker have been synthesized and their metabolic stability has been shown.

Keywords: antifiloviral agent; rat blood; HPLC-MS/MS; pharmacokinetics

Introduction



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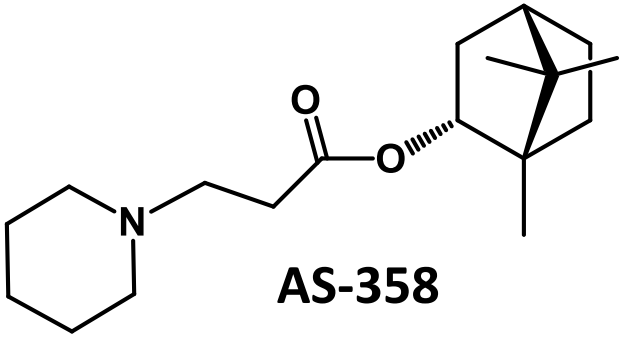


AS-358

Ebola virus
IC_{50} = 9 µM, SI=30,8

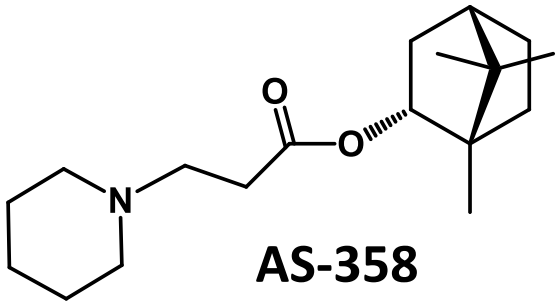
Marburg virus
IC_{50} = 9 µM, SI=35

(1*S*,2*R*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-yl 3-(piperidin-1-yl)propanoate

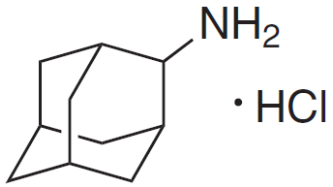
The compound showed high antifiloviral activity in *in vitro* experiments. (O. I. Yarovaya, A. S. Sokolova *et al.* Pat. RU 2 649 406, 2018 O.I. Yarovaya, A.S. Sokolova *et al.*; Patent RU 2697716, 2019)

Targets and goals

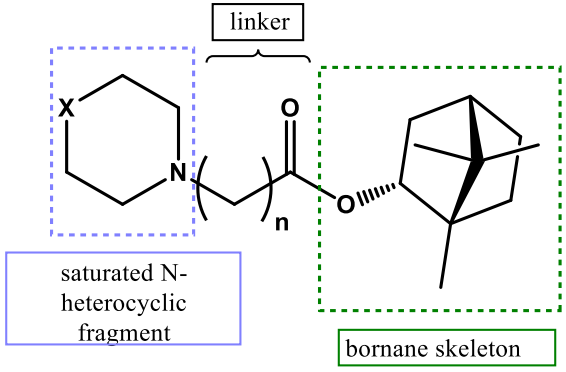
- Study of the stability of the antifiloviral agent AS-358 in rat blood and plasma
- Determination of the decomposition products of the AS-358 compound
- Development and validation of a method for the quantitative determination of AS-358 in rat whole blood
- Study of the pharmacokinetics of the compound AS-358 after oral administration to rats
- Synthesis of stable analogues of AS-358 having ether as a linker



AS-358



2-amino-adamantane hydrochloride (internal standard)



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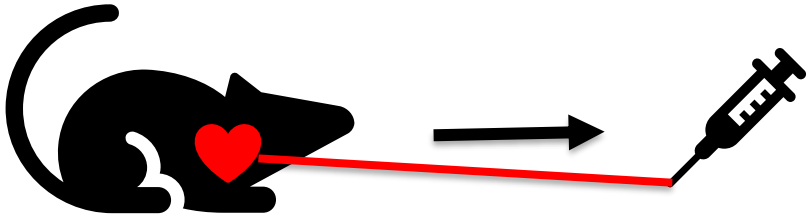
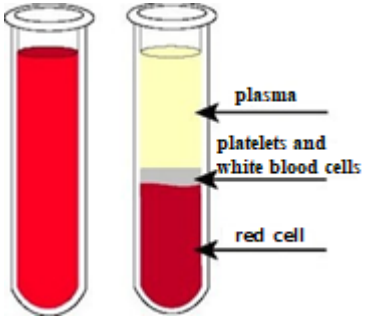
Materials, equipment, methods

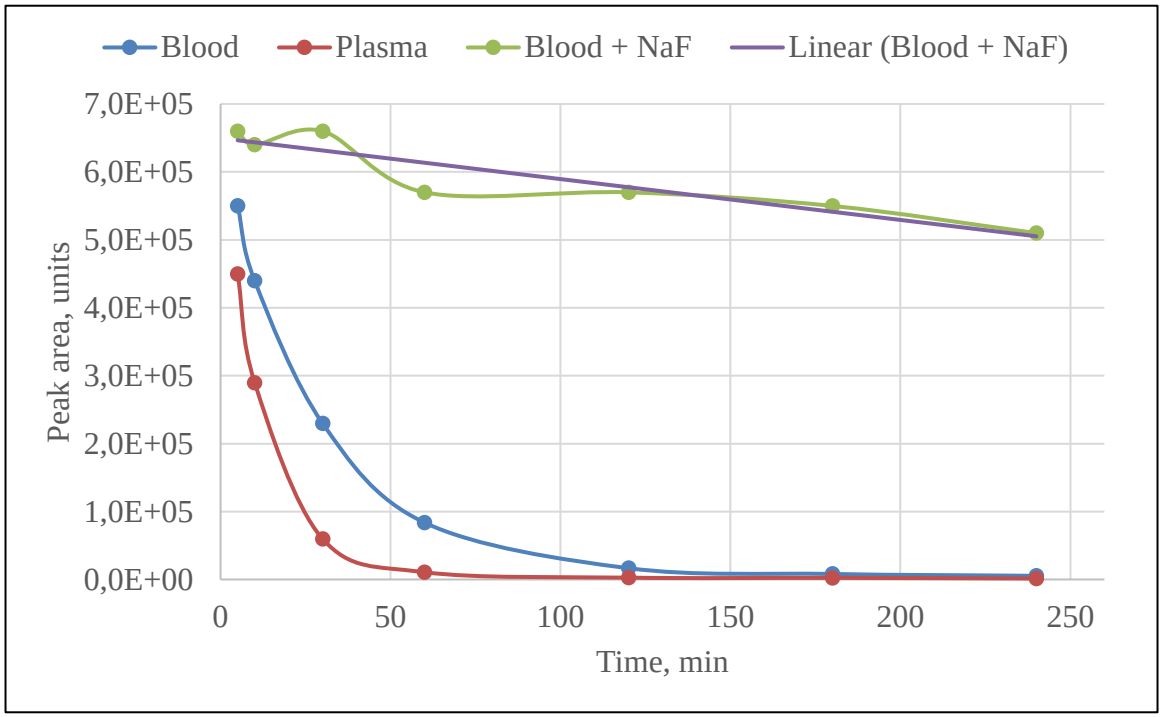




Chromatograph Shimadzu LC-20AD Prominence

Mass-spectrometer AB SCIEX 6500

Stability of AS-358 in blood and plasma *in vitro*

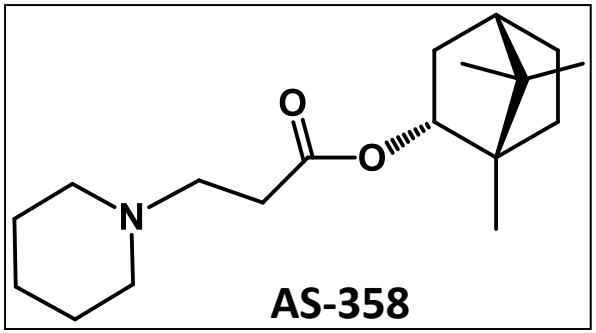


Vacutainers with blood with anticoagulants:

а) 

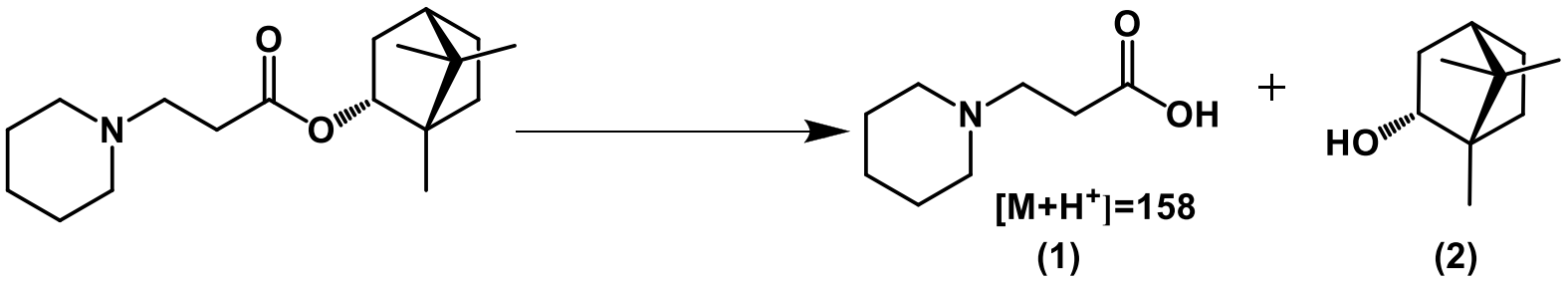
б) 

а) EDTA *б) EDTA with NaF*

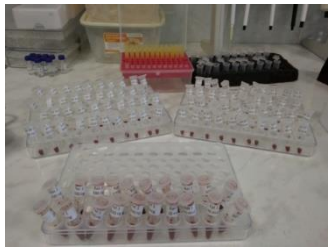


The use of NaF as an addition results in inhibition of the enzymes taking part in hydrolysis of AS-358.

Search and establishment of the structure of the metabolite formed from AS-358 in blood and plasma *in vitro*

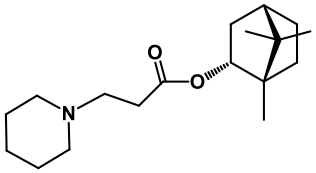
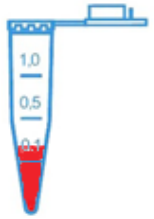


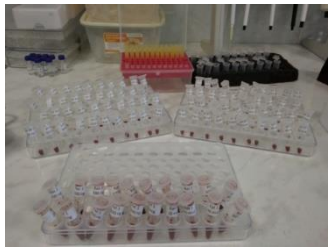
1) NaOH, MeOH, 48 ч.
2) HCl|Et_2O, pH~7, solvent stripping
3) Treatment of the mixture with hexane → borneol (2) (GC-MS)
4) Treatment CH_2Cl_2 → acid (1) + borneol (2) (GC-MS, HPLC-MS/MS)



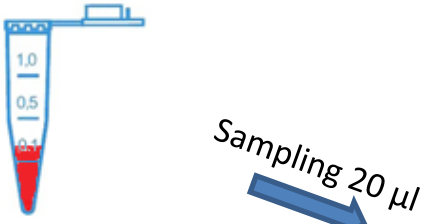
Sample preparation scheme

To prepare calibration solutions:

AS-358 (20 µl) 	(10 ng/ml) (20 ng/ml) (30 ng/ml) (50 ng/ml) (100 ng/ml) (200 ng/ml) (500 ng/ml) (1000 ng/ml) (2000 ng/ml) (4000 ng/ml) (5000 ng/ml)	+ blood with EDTA + NaF preservative (180 µl) = 	spike (200 ml) 	(1 ng/ml) (2 ng/ml) (3 ng/ml) (5 ng/ml) (10 ng/ml) (20 ng/ml) (50 ng/ml) (100 ng/ml) (200 ng/ml) (400 ng/ml) (500 ng/ml)



Sample preparation scheme

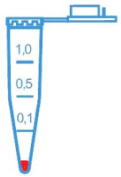


spike (200 ml)

Sampling 20 µl

Sampling 20 µl

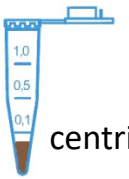
An animal that has been orally administered with AS-358



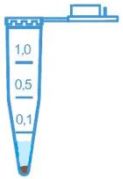
20 µl sample

50 µl 0.2M

$ZnSO_4$/MeOH



centrifugation

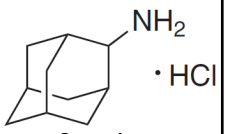


50 µl supernatant

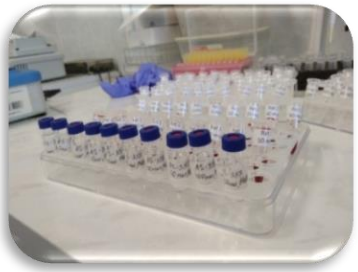
+100 µl H_2O

Quantitative determination procedure

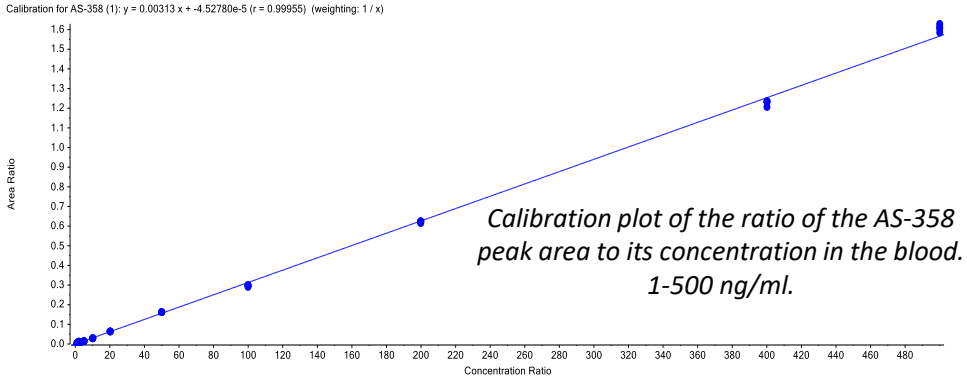
- Taking into account the stability data, a method for the quantitative determination of AS-358 in rat blood was developed and validated.
- The bioanalytical method was validated in accordance with the FDA and EMA regulations in terms of selectivity, calibration curve, accuracy, recovey, carry over and stability of a prepared sample in autosampler.



2-amino-adamantane hydrochloride (internal standard)



Calibration solutions



Calibration plot of the ratio of the AS-358 peak area to its concentration in the blood. 1-500 ng/ml.

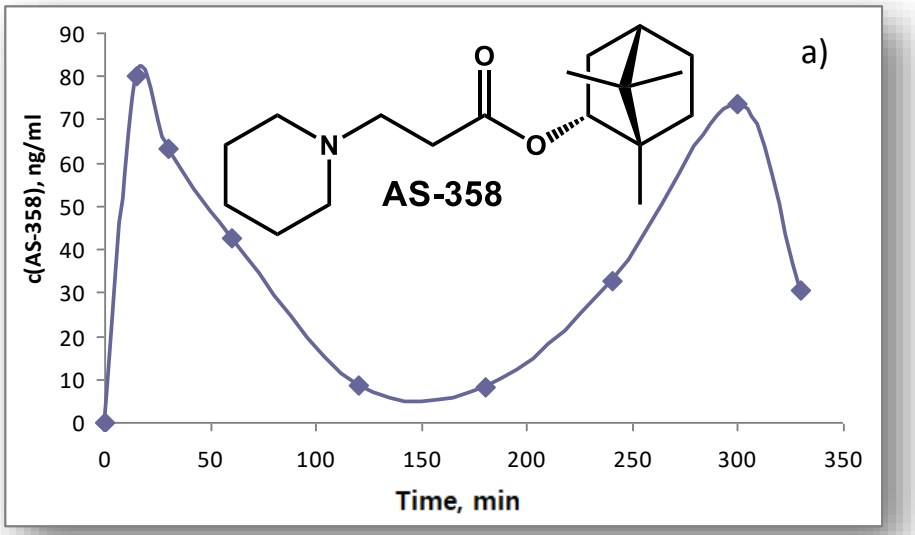
Preliminary study of pharmacokinetics

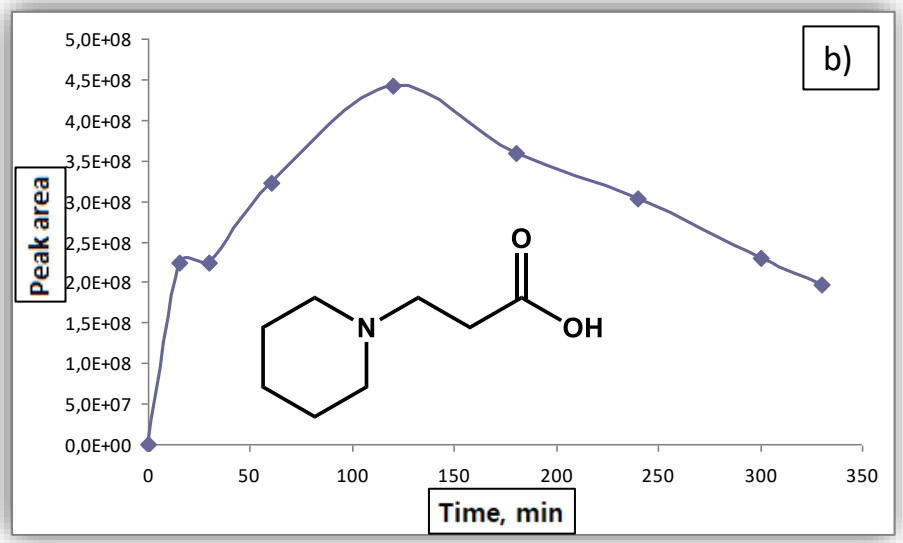
- mixture AS-358 with chalk (1:10), suspended in saline
- mixture of AS-358 with chalk (1:3), suspended in saline
- AS-358 suspension in 1% aqueous starch

Dose 200 mg/kg, 3 animals

Blood was collected from the tail vein after 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 24 h.

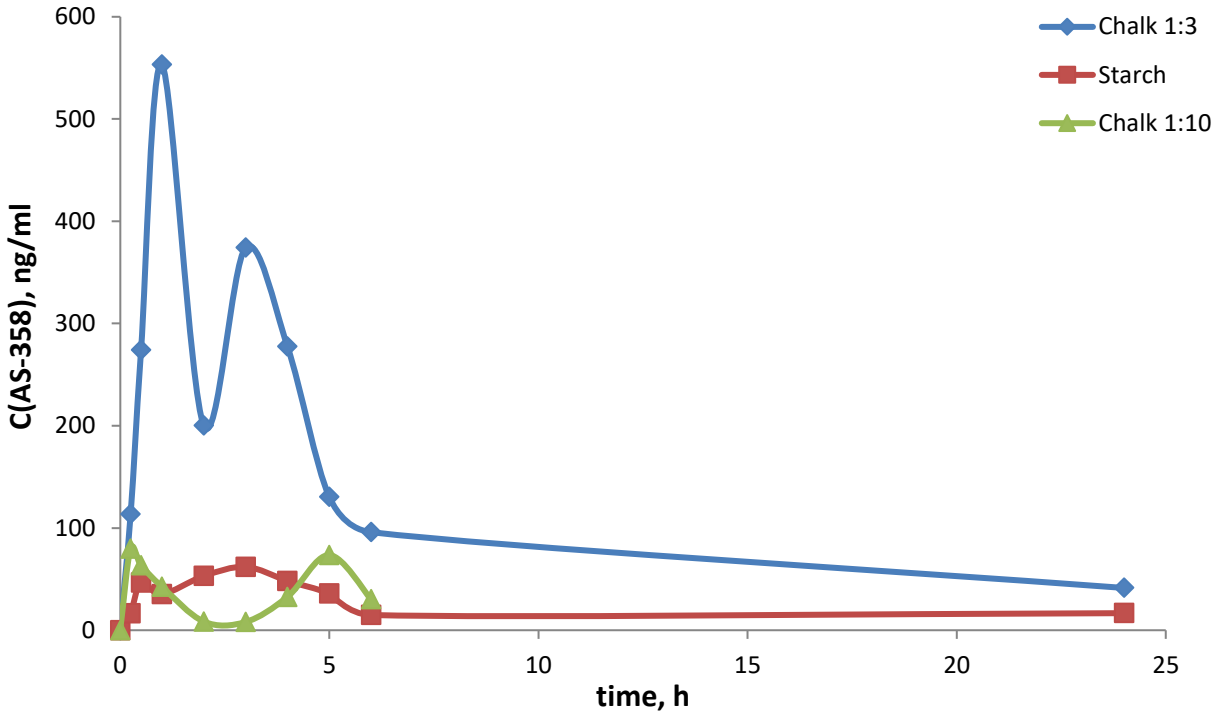
Metabolite detection





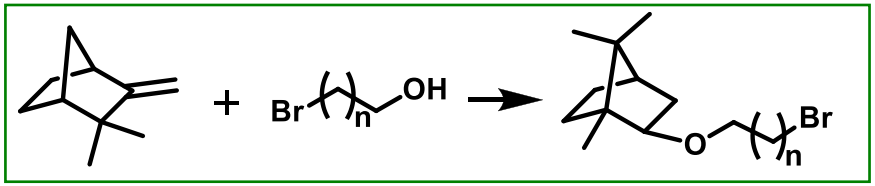
Concentration-time profile: a) compounds AS-358; b) 3- (1-piperidinyl) -propanoic acid

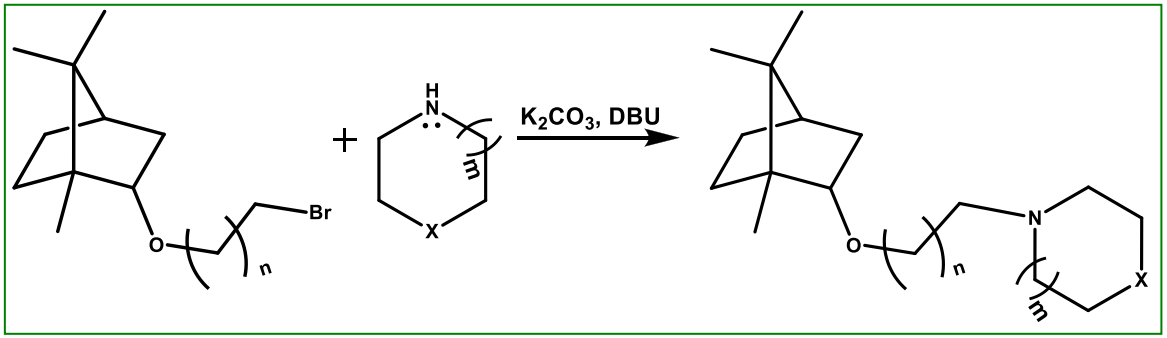
Dependence "concentration - time" of AS-358 in the blood of animals after oral administration



Synthetic part

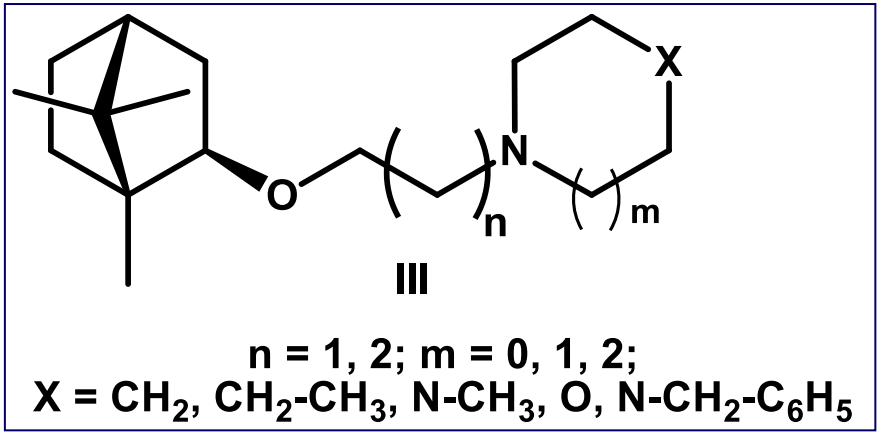
It is based on the previously developed two-stage method starting from camphene: at the first stage camphene reacts with 2-bromoethanol / 3-bromopropanol in the presence of K-10 cationoid clay producing a bromoether. This bromoether then subsequently alkylates a saturated nitrogen heterocycles leading to target compounds.





n = 1, 2; m = 0, 1, 2; X= CH_2, CH_2-CH_3, N-CH_3, O, N-CH_2-C_6H_5

Synthesis of target compounds

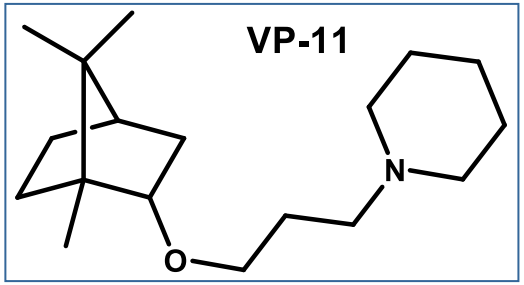


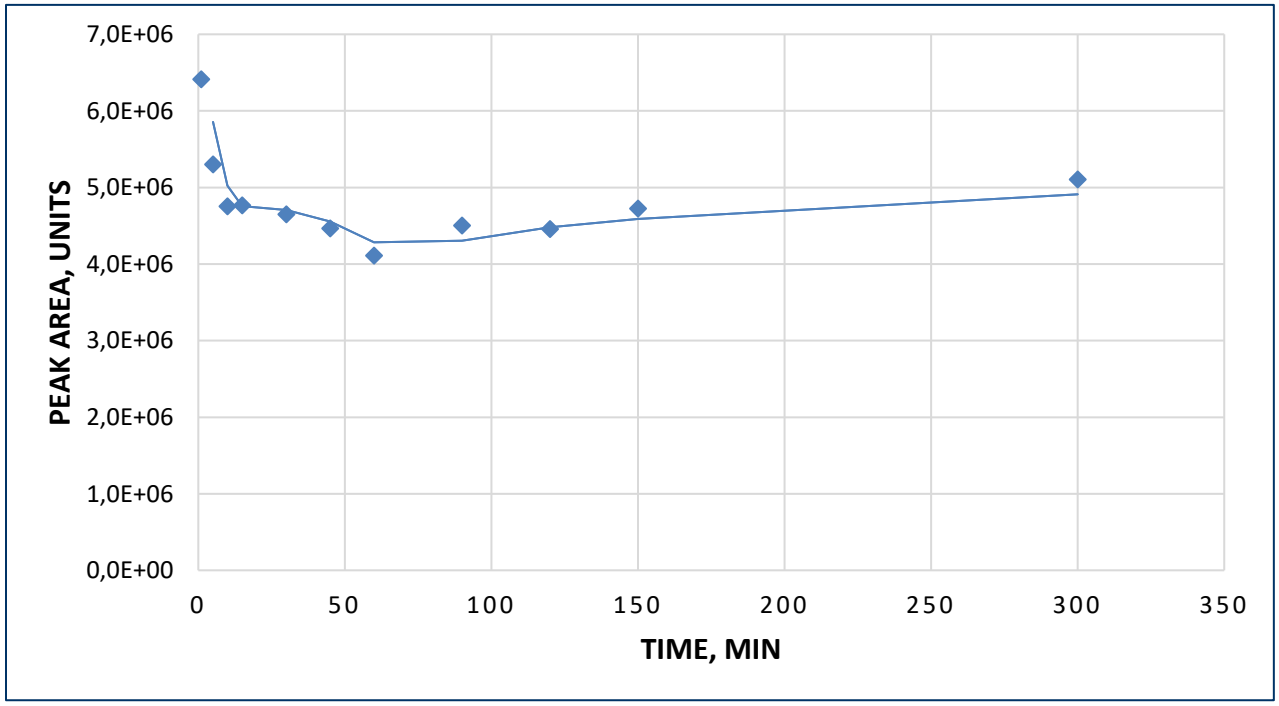
III

n = 1, 2; m = 0, 1, 2;
X = CH_2, CH_2-CH_3, N-CH_3, O, N-CH_2-C_6H_5

At the moment, all new substances are being tested at the State Research Center of Virology and Biotechnology VECTOR.

VP-11 stability check





- In rat blood, after distribution of the substance VP-11 between blood cells and plasma, no hydrolysis or other transformations are observed.
- Thus, it was shown that ethers based on borneol and 3-(piperidin-1-yl) -propane do not undergo enzymatic hydrolysis when introduced into rat blood.

Conclusions

- The stability of the agent in rat whole blood and plasma was studied.
- A method for the quantitative determination of the AS-358 agent in rat whole blood by HPLC-MS / MS was developed and validated.
- A preliminary study of the pharmacokinetics of the agent AS-358 after oral administration to rats in the form of three different dosage forms at a dose of 200 mg / kg was carried out.
- It has been shown that the use of a suitable dosage form can ensure the presence of the AS-358 agent in rat blood, despite its instability.
- The synthesis of a set of camphene derivatives containing an ether linker of various lengths and a set of saturated N-heterocyclic fragments was carried out.
- A method for the detection of agent VP-11 using HPLC-MS / MS was developed and the stability of the substance in rat whole blood was shown. The applicability of the method of sample preparation of rat blood samples using zinc sulfate precipitation for the analysis of the agent in the blood is shown, and a calibration dependence in the range of 1-1000 ng/ml is constructed.