Drug Metabolism & Disposition

Human Pharmacokinetics of LYS006, an Oral Leukotriene A4 Hydrolase Inhibitor Displaying Target-Mediated Drug Disposition

ONLINE DATA SUPPLEMENT

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*Corresponding author.

This appendix has been provided by the authors to give readers additional information about their work.
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## Results

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## Methods

### Supplementary tables

**Supp. Table S1.** Assessment schedule for PK sample collection for single ascending dose (q.d. and b.i.d.)

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<td>V101</td>
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<td>24-h fluid inputs and outputs</td>
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PK blood collection2

PK urine collection (h)  
0–2 2–4 4–8 8–12 12–24 24 h pool 24 h pool

Participants domiciled  
X  X

Meal record  
X

PK Blister fluid3  
X

Details of highly repetitive assessments for single ascending dose (q.d.)

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<td>12</td>
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<p>| V102             | 2     | 24   | X |
| V103             | 3     | 48   | X |
| V104             | 4     | 72   | X |
| V105             | 8     | 168  | X |
| V106             | 11    | 240  | X |
| EOS              | V199  | 15   | 336 | X |</p>
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<td>PK urine collection (h)</td>
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<td>2–4 h</td>
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<td>PK Blister fluid(^3)</td>
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\(^1\)Visit structure given for internal programming purpose only.
\(^2\)Assessment of the participant was required to return to complete the in case of discontinuation of study treatment or who decide they did not wish to participate in the study further.
\(^3\)Applicable only in selected cohort. Cantharidin application was done within 24 h (\(\pm 1\) h) prior to fluid sampling.
\(^4\)The participant was required to return to complete assessments in case of discontinuation of study treatment or who decided they did not wish to further participate in the study.
\(^5\)\(\pm 30\) minutes.

b.i.d., twice daily; EOS, end of study; h, hour; PK, pharmacokinetics; q.d., once daily.
### Supp. Table S2. Assessment schedule for PK sample collection for food effect

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<th>Period 1 / Period 2</th>
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<td>V9⁴</td>
<td>V107, V108, V109, V110, V111, V112</td>
</tr>
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<td>Study Day(s)</td>
<td>15, 16</td>
<td>17, 18, 19, 23, 26</td>
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<td>24-h fluid inputs and outputs</td>
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</tr>
<tr>
<td>PK blood collection²</td>
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<td>PK urine collection (h)</td>
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<td>Meal record</td>
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</table>

¹Visit structure given for internal programming purpose only. ²Assessment of the participant was required to return to complete in case of discontinuation of study treatment or who decide they do not wish to participate in the study further. ³Baseline, ⁴Baseline - The two dosing days were separated by a wash-out period of at least 14 days.

EOS, end of study; h, hour; m, minute; PK, pharmacokinetic.
**Supp. Table S3.** Assessment schedule for PK sample collection for multiple ascending dose (q.d.)

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<tr>
<td>PK urine collection (h)</td>
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<td>0–2 2–4 4–8 8–12 12–24 24 h pool 24 h pool</td>
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## Details of highly repetitive assessments

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1. Visit structure given for internal programming purpose only.
2. Assessment the participant was required to return to complete in case of discontinuation of study treatment or who decided they do not wish to participate in the study further.
3. The first cohort was dosed under fasted conditions. Second cohort and subsequent additional cohorts were dosed under fed or faster conditions.
4. Applicable only in selected cohorts. Cantharidin application was done within 24 h (+/- 1h) prior to fluid sampling (at Baseline for Day 1 sample collection and on Day 8 for Day 9 collection). The sample collection was done before dosing.
5. Applicable only in selected cohorts. The skin biopsy was collected before dosing.
6. Assessment the participant should have returned to complete in case of discontinuation of study treatment or who decided they do not wish to participate in the study further.

EOS, end of study; PK, pharmacokinetics; q.d., once daily.
**Supp. Table S4. Comprehensive list of inclusion and exclusion criteria**

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<th>Exclusion criteria</th>
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<td>Written informed consent</td>
<td>Use of other investigational drugs at enrollment/within 5 half-lives of enrollment/within 30 days</td>
</tr>
<tr>
<td>Healthy participants aged 18–45 years</td>
<td>A history of clinically significant ECG abnormalities</td>
</tr>
<tr>
<td>Participants in good health (as determined by past medical history, physical examination, vital signs, ECG, and laboratory tests at screening)</td>
<td>Known history/family history of long QT syndrome</td>
</tr>
<tr>
<td>Participants weighing ≥50 kg with a BMI within the 18–30 kg.m(^2) range</td>
<td>Know history of current clinically significant arrhythmias</td>
</tr>
<tr>
<td>At screening, vital signs (SBP, DBP and pulse rate) were assessed in the supine position after the participant rested for at least five minutes, and again (when required) after three minutes in the standing position. Sitting vital signs were required to be within the normal ranges or the following ranges: oral body temperature between 35.0–37.5 °C, SBP, 90–140 mmHg, DBP, 50–90 mmHg, pulse rate, 40–90 bpm.</td>
<td>History/presence of crystals or stones in urine (at screening/baseline)</td>
</tr>
<tr>
<td>Ability to communicate well with the investigator and understand/comply with the requirements of the study</td>
<td>History of malignancy/precancerous condition of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of evidence of local recurrence or metastases.</td>
</tr>
<tr>
<td></td>
<td>Pregnant or lactating women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test</td>
</tr>
<tr>
<td></td>
<td>Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant</td>
</tr>
<tr>
<td></td>
<td>Sexually active males who did not use a condom during intercourse while taking the drug and for 15 days after stopping study medication and fathered a child during this period. A condom was required to be used also by vasectomized men to prevent delivery of the drug via seminal fluid</td>
</tr>
<tr>
<td></td>
<td>Used any prescription drugs, herbal supplements, within four weeks prior to initial dosing, and/or over-the-counter medication, dietary supplements within two weeks prior to initial dosing</td>
</tr>
<tr>
<td></td>
<td>Donation or loss of 400 mL or more of blood within eight weeks prior to initial dosing, or longer if required by local regulation</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin level &lt;12.0 g/dL at screening/baseline</td>
</tr>
<tr>
<td></td>
<td>Significant illness that has not resolved within two weeks prior to initial dosing</td>
</tr>
<tr>
<td></td>
<td>Active systemic infections (other than common cold) during the two weeks prior to Baseline</td>
</tr>
<tr>
<td></td>
<td>Recent and/or recurrent history of autonomic dysfunction</td>
</tr>
<tr>
<td></td>
<td>Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the participant in case of participation in the study</td>
</tr>
<tr>
<td></td>
<td>Flu-like symptoms within two weeks prior to dosing</td>
</tr>
<tr>
<td></td>
<td>History of immunodeficiency diseases, including a positive HIV test result</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Exclusion criteria</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chronic infection with HBV or HCV. A positive HBV surface antigen test, or if</td>
<td>Chronic infection with HBV or HCV. A positive HBV surface antigen test, or if standard local practice, a positive HBV core antigen test, would exclude a participant. Participants with a positive HCV antibody test should have HCV RNA levels measured. Participants with positive HCV RNA should be excluded</td>
</tr>
<tr>
<td>standard local practice, a positive HBV core antigen test, would exclude a</td>
<td>Positive QuantiFERON-TB Gold test</td>
</tr>
<tr>
<td>participant. Participants with a positive HCV antibody test should have HCV RNA</td>
<td>BCG vaccination in the six weeks preceding baseline</td>
</tr>
<tr>
<td>levels measured. Participants with positive HCV RNA should be excluded</td>
<td>Smokers. Urine cotinine levels would be measured during screening/baseline. Smokers were defined as any participant who reported tobacco use and/or who has a urine cotinine ≥500 ng/mL</td>
</tr>
<tr>
<td>Positive QuantiFERON-TB Gold test</td>
<td>Any clinically significant white blood cell count or clinically significant white blood cell lab abnormalities at screening/baseline</td>
</tr>
<tr>
<td>BCG vaccination in the six weeks preceding baseline</td>
<td>History of allergy to the investigational compound class being used</td>
</tr>
<tr>
<td>Smokers. Urine cotinine levels would be measured during screening/baseline.</td>
<td>History of hypersensitivity to any of the study drugs (including local anesthesia) or to drugs of similar chemical classes</td>
</tr>
<tr>
<td>Smokers were defined as any participant who reported tobacco use and/or who has</td>
<td>Recent (within the last three years) and/or recurrent history of acute or chronic bronchospastic disease (including asthma or aspirin exacerbated respiratory disease and chronic obstructive pulmonary disease, treated, or not treated).</td>
</tr>
<tr>
<td>a urine cotinine ≥500 ng/mL</td>
<td>History of drug abuse/unhealthy alcohol use within the 12 months prior to dosing, or evidence as indicated by the laboratory assays</td>
</tr>
<tr>
<td>Any clinically significant white blood cell count or clinically significant white</td>
<td>Presence of chronic skin inflammatory diseases (such as psoriasis) or skin infection with anterior arm involvement not compatible with skin application of cantharidin</td>
</tr>
<tr>
<td>blood cell lab abnormalities at screening/baseline</td>
<td>Presence of large tattoos on the arm not compatible with skin application of cantharidin</td>
</tr>
<tr>
<td>History of allergy to the investigational compound class being used</td>
<td>History of hypertrophic scarring (for MAD only)</td>
</tr>
</tbody>
</table>

BCG, Bacillus Calmette Guerin; BMI, body mass index; DBP, diastolic blood pressure; ECG, electrocardiogram; HBV, hepatitis B virus; HCG, human chorionic gonadotrophin; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MAD, multiple ascending dose; RNA, ribonucleic acid; SBP, systolic blood pressure; TB, tuberculosis.
S1. Metabolite analysis and drug disposition

S1.1 LC-MS/MS conditions for metabolite profiling

Ultra-performance liquid chromatography (UHPLC) was performed on an ACQUITY system (Waters Corp., Milford, MA, US). The separation was carried out on an ACQUITY UHPLC HSS T3 column (150 mm × 2.1 mm i.d., 1.8 µm; Waters Corp., Milford, MA, US), protected with a ACQUITY UHPLC HSS T3 precolumn (2.1 x 5 mm, 1.8 µm). The precolumn and the column temperatures were maintained at 50°C. The analysis was achieved with a gradient elution using A (10 mM ammonium acetate [Fluka, St-Louis, US] in water [Optima™ LC/MS Grade; Fisher Chemical, Loughborough, UK] at pH 5.5, adjusted with ammonia [Merck, Darmstadt, Germany]) and B acetonitrile (Fisher Chemical, Loughborough, UK) as the mobile phase. The gradient condition, with a flow rate at 0.5 mL/min., was: 0–1 min 5% B; 1–2 min, linear from 5% to 15% B; 2–18 min, linear from 15% to 28% B; 18–30 min, linear from 28% to 60% B; 30–35 min, linear from 60% to 100% B held at 100% B for 6 min and then an immediate reduction to 5% B for equilibration of the column. The Waters ACQUITY Synapt G2-Si Mass Spectrometer (Waters Corp., Manchester, UK) was coupled to the UHPLC system via an electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode with a capillary voltage at 3.0 kV and a cone voltage at 40V. The temperature of the source and desolvation was set at 120°C and 150°C, respectively. The desolvation gas flow rate was 800 L/h and the cone gas flow rate was 25 L/h. All data collected in centroid mode were acquired using Masslynx V4.1 software (Waters Corp., Milford, MA, US). A solution of leucine-enkephalin (Sigma-Aldrich Chemie, Steinheim, Germany) was infused through the LockSpray interface to generate a lock-mass throughout the analysis and obtain accurate mass spectrometry data.

S1.2 Semi-quantification of M3 and M4 using synthetic reference standards

A stock solution containing synthetic reference standards of M3 and M4 at 1,000 µM was prepared in DMSO (Acros Organics, Geel, Belgium). Blank human plasma (obtained from pre-dose samples from same participants) was added to the stock solution to prepare plasma calibration samples at 2,000, 200, 50, 20, 5 and 2 nM by serial dilution. Blank human urine (obtained from placebo group from same cohort) was added to a 100 µM stock solution of synthetic reference standards in DMSO to prepare urine calibration samples at 500, 200, 50 and 20 nM by serial dilution. Calibration samples were processed together with
test samples as described in the sample preparation section before injection on the LC-MS/MS system for analysis.

S1.3 Semi-quantification of M1 and M6 using $[^3$H$]$ radiolabeled standards from a rat ADME study

Biologically derived $[^3$H$]$ metabolites from rat urine collected during a preclinical rat $[^3$H$]$ radiolabeled ADME study were used as $[^3$H$]$ metabolite standards. The concentration of each standard in rat urine was calculated based on radioactivity counting. The amount of metabolites M1 and M6 in human plasma pool were estimated by determining relative MS responses of metabolites between plasma and $[^3$H$]$ metabolite standards, and using the calculated concentrations of metabolite standards as calibrants. The detailed methodology and calculations were described by Yu et al., 2007. Rat urine pool 0–48 h from a rat $[^3$H$]$ radiolabeled ADME study, where animals were dosed i.v. with 1 mg/kg LYS006, was used to prepare radioactive calibration samples for the quantification of LYS006 metabolites M1 and M6 in human plasma and urine samples. Five calibration concentrations were prepared by 1, 10-, 20-, 100- and 1000-fold dilutions of the rat urine containing the $[^3$H$]$ metabolite standards with blank rat urine. For human plasma test sample, in order to matrix match the samples, blank rat urine was added in a ratio of 1:1 (v:v) to the human plasma AUC$_{0-24h}$ pool before extraction. For the plasma calibration samples, blank human plasma (obtained from placebo group from same cohort) was added in the same 1:1 (v:v) ratio to each radioactive rat urine calibration samples before extraction. For the human urine test sample, in order to matrix match the samples, blank rat urine was added in a ratio of 1:1 (v:v) to the human 0–24 h urine pool. For the urine calibration samples, blank human urine (obtained from placebo group from same cohort) was added in the same 1:1 (v:v) ratio to each radioactive rat urine calibration samples. Then, samples were processed, and internal standard was added as described in the sample preparation section before injection on the LC-MS/MS system for analysis.

S2. In vitro characterization of LYS006 as a transporter substrate

All in vitro experiments were conducted at a clinical research organization (SOLVO biotechnology, Szeged, Hungary). The purpose of the transporter analysis was to investigate if LYS006 was a substrate of the human ABC (efflux) transporters BCRP, P-gp (MDR1), MRP2, and MRP4 as well as if LYS006 was a substrate of the human SLC (uptake) transporters OAT1, OAT3, OAT4, and OCT2.
S2.1 Materials

LYS006 was obtained from Novartis Pharma AG (Basel, Switzerland). The molecular mass is 392.78 g/mol and the drug content of the batch used in this study was 100%. A 20 mM stock solution of LYS006 was prepared in dimethyl sulfoxide (DMSO, Merck KGaA, Darmstadt, Germany) and stored at ≤15°C. 

[3H]-LYS006 was obtained from RC Tritec (Teufen, Switzerland) as a 1 mCi/mL (37 MBq/mL; 16.30 µg/mL) ethanol solution and was stored at < -60°C. The radiochemical purity of the used lot was >99%.

Radiolabeled reference compounds [14C]-metformin and [14C]-uric acid were obtained from Moravek (Brea, CA, US). The radiolabeled reference compounds [3H]-para-aminohippuric acid, [3H]-dehydroepiandrosterone sulfate, [3H]-estradiol-17-β-glucuronide, [3H]-digoxin, and [3H]-prazosin were from Perkin Elmer (Waltham, MA, US). [3H]-Estrone-3-sulfate was from the radiolaboratory of the Biological Research Center (Szeged, Hungary). All other chemicals were purchased from commercial sources and were of analytical grade.

S2.2 Cell lines

Human embryonic kidney 293 (HEK293) cell lines overexpressing the human uptake transporters OAT1, OAT2, OAT4, or OCT2 were generated by SOLVO Biotechnology (Szeged, Hungary) using lentiviral transduction of HEK293 cells with the corresponding transporter. Madin-Darby canine kidney II (MDCKII) cell lines overexpressing the human efflux transporters P-gp and BCRP were generated by SOLVO Biotechnology (Szeged, Hungary) using lentiviral transduction of MDCKII cells with either transporter (Invitrogen, Waltham, MA, US). Transduced and antibiotic-selected cells were tested for transporter-specific uptake activity and stability of expression (Tátrai et al., 2019).

S2.3 Membrane vesicles

Inside-out membrane vesicles prepared from HEK293 cells, which stably overexpress the human ABC transporters MRP2 or MRP4, were used from SOLVO Biotechnology (Szeged, Hungary).

S2.4 In vitro uptake (SLC) transporter phenotyping (OAT1, OAT3, OAT4, OCT2)
Cells were cultured at 37±1°C in an atmosphere of air: CO₂ (95:5) and were plated onto standard 24-well tissue culture plates at the density 5 × 10⁵ cells/well using Dulbecco’s modified Eagle’s medium (DMEM), 4500 mg/l of glucose, supplemented with GlutaMAX, 10% v/v fetal bovine serum (FBS) and 1% v/v penicillin-streptomycin (all from Gibco/ThermoFisher Waltham, MA, US).

The uptake of LYS006 was determined using cells overexpressing the respective uptake transporter (OAT1, OAT3, OAT4, and OCT2) and mock transfected control cells, at a single incubation time point (5 min) and at ten concentrations of LYS006 in a range of 0.25–200 µM. The transporter-specific uptake of LYS006 was determined in the presence of a known inhibitor of the respective transporters at a LYS006 concentration of 0.25 µM (Table S5).

Before the experiment, the medium was removed and the cells were washed twice with 300 µL of assay buffer (Henseleit-Krebs buffer at pH 7.4 for OAT3 or Hank’s Buffered Salt Solution buffer at pH 7.4 for OAT1, OAT4, and OCT2, all from Gibco/ThermoFisher Waltham, MA, US). Cellular uptake of LYS006 into the cells was measured by adding 300 µL of assay buffer containing LYS006 and incubating them at 37±1°C. Reactions were stopped by removing the assay buffer and the cells were washed twice with 300 µL assay buffer. Cells were lysed by adding 300 µL of 0.1 M NaOH and incubated for 10 minutes at 37±1°C. The amount of [³H]-LYS006 in the cell lysate was determined by liquid scintillation counting. The amount of protein in each well was quantified using the BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, US). Parameters of the uptake transporter assays are presented in Table S5.

Supp. Table S5. Parameters of uptake transporter assays

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Incubation time (min)</th>
<th>Probe substrate</th>
<th>Reference inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>human OCT2</td>
<td>2</td>
<td>Metformin (10 µM)</td>
<td>Verapamil (300 µM)</td>
</tr>
<tr>
<td>human OAT1</td>
<td>2</td>
<td>PAH (5 µM)</td>
<td>Benz bromarone (300 µM)</td>
</tr>
<tr>
<td>human OAT3</td>
<td>1</td>
<td>E3S (1 µM)</td>
<td>Probenecid (500 µM)</td>
</tr>
<tr>
<td>human OAT4</td>
<td>5</td>
<td>Uric acid (20 µM)</td>
<td>Benz bromarone (300 µM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diclofenac (100 µM)</td>
</tr>
</tbody>
</table>

OAT, organic anion transporter; OCT, organic cation transporter.
S2.5 In vitro vesicular based efflux (ABC) transporter phenotyping (MRP2, MRP4)

The uptake of LYS006 into membrane vesicles was determined using inside-out membrane vesicles (total protein: 50 μg/well) prepared from cells overexpressing human MRP2 or MRP4. One incubation time point (8 min) and eleven concentrations of LYS006 in the range of 0.1–150 μM were tested in the presence of ATP or AMP (both from Sigma-Aldrich, St. Louis, MO, US) to determine whether the compound is actively transported into the vesicles. The assay buffers used were 46.5 mM MOPS-Tris buffer pH 7.0 containing 65 mM KCl and 7 mM MgCl₂ (MRP2) and 10 mM Tris-HCl pH 7.4 containing 250 mM sucrose and 10 mM MgCl₂ (MRP4). 4 mM Mg-ATP or 4 mM AMP were added to start the reactions. Reactions were quenched by the addition of 200 μL of ice-cold washing buffer (40 mM MOPS-Tris/70 mM KCl [MRP2] and 10 mM Tris-HCl/250 mM sucrose/100 mM NaCl containing 0.02% w/v bovine serum albumin [MRP4], all from Gibco/ThermoFisher Waltham, MA, US) and immediately filtrated through glass fiber filters mounted to a 96-well plate (filter plate, MultiScreen Harvest APFC, Merck KGaA, Darmstadt, Germany). The filters were washed five times with 200 μL of ice-cold washing buffer and subsequently dried. The amount of accumulated [³H]-LYS006 retained inside the vesicles was determined by liquid scintillation counting. Parameters of the vesicular transporter assays are presented in Table S6.

Supp. Table S6. Parameters of the vesicular transport assay

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Protein content / well (µg)</th>
<th>Incubation time (min)</th>
<th>Probe substrate</th>
<th>Reference inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>human MRP2</td>
<td>50</td>
<td>8</td>
<td>E217βG (50 µM)</td>
<td>BB (100 µM)</td>
</tr>
<tr>
<td>human MRP4</td>
<td>50</td>
<td>8</td>
<td>DHEAS (0.5 µM)</td>
<td>MK571 (150 µM)</td>
</tr>
</tbody>
</table>

BB, Benzbromarone; DHEAS, Dehydroepiandrosterone sulfate; MK571, 5-(3-(2-(7-Chloroquinolin-2-yl)ethenyl)phenyl)-8-dimethylcarbamyl-4,6-dithiaoctanoic acid sodium salt hydrate, MRP, multidrug resistance-associated protein.

S2.6 In vitro monolayer efflux (ABC) transporter phenotyping (P-gp, BCRP)

The monolayer assays were performed using BCRP or MDR1 transfected MDCKII cell monolayers. MDCKII-BCRP and MDCKII-MDR1 cells were cultured in Dulbecco’s Modified Eagle’s Medium with 4.5 g/L glucose (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (all from Gibco/ThermoFisher Waltham, MA, US) at 37°C in an atmosphere of air: CO₂ (95:5) in cell culture flasks prior to
seeding into 24-transwell monolayer plate inserts (Corning-Merck KGaA, Darmstadt, Germany).

Transfected MDCKII cells were cultured on the inserts with 100 µL medium per well on the apical side and 25 mL in a single-well receiver tray for all 24 wells on the basolateral side, for 96 h. Medium was changed 24 h before the experiment. Before the experiment cells were washed twice with the HBSS assay buffer and then pre-incubated in HBSS buffer pH 7.4 (Hank's Buffered Salt Solution with carbonate, Gibco/ThermoFisher Waltham, MA, US) for 1 h to allow cells adjusting to the medium. HBSS buffer containing LYS006 at ten concentrations in a range between 0.5–300 µM was then added to the appropriate apical (400 µL) or basolateral chamber (800 µL). The final concentration of DMSO (Merck KGaA, Darmstadt, Germany) in the incubations did not exceed 1% (v/v). The efflux ratio of prazosin and digoxin was determined as a positive control for BCRP and MDR1 function, respectively.

After the incubation at 37±1°C, aliquots (100 µL) were taken from the receptor chambers to determine the amount of translocated LYS006 and controls. Samples were taken from the donor chambers before and after incubation to determine the initial concentration (C₀) and recovery (R) of the test compound and the controls. Bidirectional transport of LYS006 in MDCKII-BCRP and MDCKII-MDR1 cells was determined by liquid scintillation counting.

Bidirectional transport of LYS006 in MDCKII-BCRP and MDCKII-MDR1 was also determined in the presence and absence of BCRP inhibitor Ko143 (Sigma-Aldrich, St. Louis, MO, US) and MDR1 inhibitor LY335979 (Carbosynth Ltd, Berkshire, UK) individually and mixed together to confirm the specificity of the transport in MDCKII-BCRP and MDCKII-MDR1 cells. The transported compound was applied at one selected concentration (0.5 µM) and incubation time (120 min). The effect of the reference inhibitors was studied at one concentration (1 µM Ko143, 5 µM LY335979 and Ko143+LY335979 as a cocktail).

Parameters of the monolayer transporter assays are presented in Table S7.

**Supp. Table S7.** Parameters of monolayer transporter assays

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Incubation time (min)</th>
<th>Probe substrate</th>
<th>Reference inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>human P-gp</td>
<td>120</td>
<td>Digoxin (5 µM)</td>
<td>LY335979 (5 µM)</td>
</tr>
<tr>
<td>human BCRP</td>
<td>120</td>
<td>Prazosin (1 µM)</td>
<td>Ko143 (1 µM)</td>
</tr>
</tbody>
</table>

BCRP, breast cancer resistant protein; P-gp, p-glycoprotein.
S2.7 Data analysis

Radioactivity of [3H]-LYS006 and radiolabeled reference compounds in samples from in vitro transporter assays was analyzed using a Perkin Elmer MicroBeta\(^2\) liquid scintillation counter (Perkin Elmer, Waltham MA) and a BMG Labtech FluoStar Optima fluorescence photometer (BMG Labtech, Offenburg, Germany). Based on the specific activity, compound concentrations were determined, and kinetic parameters were calculated.

S2.8 Cellular uptake and vesicular transporter substrate assays

Based on the specific activity, compound amounts in the cell and vesicle lysates were determined and normalized by the protein amount, the incubation time, and the applied substrate concentration in order to obtain the apparent uptake permeability (PS\(_{\text{app}}, \text{nL/min/mg protein}\)).

In the cellular uptake assays, the accumulation of LYS006 in untransfected control cells was subtracted from the corresponding data in transporter-transfected cells. Similarly, in the vesicular transport substrate assays, the ATP-independent accumulation of LYS006 was subtracted from the ATP-dependent accumulation.

When the corrected concentration-dependent PS\(_{\text{app}}\) data are presented against the corresponding substrate concentration S (µM), the kinetic uptake parameters are reflected by the Michaelis-Menten equation (Sasaki et al., 2004):

\[
PS_{\text{app}} = PS_m + PS_c = PS_m + \frac{V_{c,\text{max}}}{K_m + S} = PS_m + \frac{PS_{c,\text{max}} \times K_m}{K_m + S} \quad (\text{Eq. S1})
\]

Where PS\(_m\), PS\(_c\) and PS\(_{c,\text{max}}\) are the passive, carrier-mediated (saturable) and maximum carrier mediated uptake permeability (nL/min/mg protein), respectively, \(V_{c,\text{max}}\) is the maximum transporter uptake velocity rate (fmol/min/mg protein), and \(K_m\) is the Michaelis-Menten constant (µM).

Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA) was used for basic data processing and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA) was used for curve fitting and determination of reaction parameters.
**S2.9 Permeability calculation in monolayer transport assays**

The following equation was used to calculate apparent permeability coefficient ($P_{app}$, cm/s):

$$P_{app} = \frac{\frac{dQ}{dT}}{A \times C_0} \quad (Eq. S2)$$

dQ is the amount of transported test drug in the acceptor compartment (pmol), dT the incubation time (s), A refers to the surface of porous membrane in cm$^2$ (standard: 0.7) and C$_0$ is the initial concentration of the compound in the donor compartment (µM).

Kinetic transport parameters were estimated from the Michaelis-Menton equation (Sasaki et al., 2004),

$$P_{app} = P_m \pm P_c = P_m \pm \frac{V_{c,\text{max}}}{K_m + S} = P_m \pm \frac{P_{c,\text{max}} \times K_m}{K_m + S} \quad (Eq. S3)$$

where $P_m$, $P_c$ and $P_{c,\text{max}}$ are the passive, carrier-mediated (saturable) and maximum carrier mediated permeability (cm/s), respectively, $V_{c,\text{max}}$ is the maximum transporter uptake velocity rate (nmol/s/cm$^2$), and $K_m$ is the Michaelis-Menten constant (µM).

Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA) was used for basic data processing and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA) was used for curve fitting and determination of reaction parameters.

**S3. LYS006 quantification**

**S3.1 Materials**

LYS006 and [13C$_2$D$_4$]LYS006 (internal standard) were obtained from Novartis Pharma AG (Basel, Switzerland). Blank plasma, urine and blister fluid were provided by Bioreclamation IVT (Westbury, NY, US), and blank blood was provided by Biopredic International (Saint-Grégoire, France). All other chemicals were purchased from commercial sources and were of analytical grade.

**S3.2 Sample preparation methods for LYS006 quantification**

The plasma sample (50 µL) preparation consisted of the addition of the internal standard ([13C$_2$D$_4$]LYS006), a protein-precipitation with acetonitrile (VWR, Radnor, PA, US), centrifugation, evaporation of the supernatant, and reconstitution of the residues with 200 µL of acetonitrile/water (MilliQ Water, Millipore,
Burlington, MA, US) (2:8; v/v) containing 0.1% of formic acid (Sigma-Aldrich, St. Louis, MO, US). 10 µL were injected onto the LC-MS/MS system.

In order to prevent adsorption, urine contained 1% Tween® 20 (Merck, Darmstadt, Germany). The urine sample (50 µL) preparation consisted of the addition of the internal standard, dilution with acetonitrile, centrifugation, and further dilution with water containing 0.1% of formic acid. 5 µL were injected onto the LC-MS/MS system.

The blood and blister fluid samples were prepared as plasma samples, as described above.

The skin biopsies samples (between 5 and 15 mg, weighted) previously crushed using a Cryoprep system CP02 (Covaris, Woburn, MA, US) were added to 50 µL blank plasma, then prepared as plasma samples, as described above.

S3.3 LC–MS/MS conditions for LYS006 quantification

High-performance liquid chromatography (HPLC) was performed on a Shimadzu system (Shimadzu Scientific Instruments, Columbia, MD, US). The separation was carried out on a XBridge C18 column (50 mm × 2.1 mm i.d., 2.5 µm; Waters Corp., Milford, MA, US) maintained at 50°C. The analysis was achieved with a gradient elution using A (water containing 0.1% of formic acid) and B (acetonitrile containing 0.1% of formic acid) as the mobile phases. The gradient condition, with a flow rate at 400 µL/min., was: 0–0.2 min 20% B; 0.2–0.7 min, linear from 20% to 30% B; 0.7–2.5 min 30%; 2.5–2.6 min, linear from 30% to 80%; 2.6–3.4 min 80% B; 3.4–3.5 min, linear from 80% to 20% B; and finally, 3.5–5.0 min 20%. The API4000 Mass Spectrometer (AB Sciex, Concord, ON, Canada) was coupled to the HPLC system via a Turbo IonSpray interface, operated in positive ionization mode with a capillary voltage at 5.0 kV. The temperature of the source was set at 600°C. The collision energy was set at 18 eV, and the following transitions were monitored: for LYS006, m/z 393.0 → m/z 365.1 (200 ms dwell time); for [13C2D4]LYS006, m/z 399.0 → m/z 371.1 (200 ms dwell time). All data was acquired using the Analyst 1.6.3 software (AB Sciex, Concord, ON, Canada).
S3.4 Method validations for plasma and urine

The bioanalytical methods were fully validated according to current FDA and EMA bioanalytical guidelines as quantitative methods in terms of linearity, specificity, accuracy, precision, recovery, matrix effect, and stability. For plasma, the calibration range was from 0.200 ng/mL to 500 ng/mL with a lower limit of quantification (LLOQ) of 0.200 ng/mL (linear regression with 1/x2 weighting factor). For urine, the calibration range was from 5.00 ng/mL to 5000 ng/mL with a lower limit of quantification (LLOQ) of 5.00 ng/mL (linear regression with 1/x2 weighting factor).

S3.5 Cross-validations for blood and blister fluid

The validated method for plasma was further successfully cross-validated for human blood and blister fluid (using blank matrices), with intra-run accuracy within ±15% (±20% at LLOQ) and precision of ≤15% (≤20% at LLOQ) only. These results have thus to be considered as exploratory results.
Results

**Supp. Table S8.** Demographics and baseline characteristics of single ascending dose and multiple ascending dose

### Single Ascending Dose

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex, n (%)</th>
<th>Race, n (%)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Male</td>
<td>Female</td>
<td>White</td>
</tr>
<tr>
<td>5 mg n=6</td>
<td>33.5±8.9</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>10 mg n=6</td>
<td>32.5±9.4</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>20 mg n=6</td>
<td>33.7±7.3</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>30 mg n=6</td>
<td>34.8±6.4</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>45 mg n=6</td>
<td>35.0±6.5</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>70 mg n=5</td>
<td>31.6±6.2</td>
<td>5 (100.0)</td>
<td>0 (0.0)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td>70 mg b.i.d. n=6</td>
<td>32.7±7.2</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>100 mg b.i.d. n=6</td>
<td>28.2±6.4</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>40 mg b.i.d. (limited hydration) n=4</td>
<td>36.8±9.0</td>
<td>3 (75.0)</td>
<td>1 (25.0)</td>
<td>4 (100.0)</td>
</tr>
<tr>
<td>All LYS006 n=51</td>
<td>33.1±7.2</td>
<td>48 (94.1)</td>
<td>3 (5.9)</td>
<td>47 (92.2)</td>
</tr>
<tr>
<td>Placebo n=18</td>
<td>32.9±6.6</td>
<td>17 (94.4)</td>
<td>1 (5.6)</td>
<td>17 (94.4)</td>
</tr>
<tr>
<td>Total N=69</td>
<td>33.0±7.0</td>
<td>65 (94.2)</td>
<td>4 (5.8)</td>
<td>64 (92.8)</td>
</tr>
</tbody>
</table>

### Food effect

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex, n (%)</th>
<th>Race, n (%)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Male</td>
<td>Female</td>
<td>White</td>
</tr>
<tr>
<td>10 mg fasted/fed n=6</td>
<td>34.7±10.7</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>10 mg fed/fasted n=6</td>
<td>35.5±4.7</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>Total N=12</td>
<td>35.1±7.9</td>
<td>11 (91.7)</td>
<td>1 (8.3)</td>
<td>11 (91.7)</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

### Multiple Ascending Dose

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex, n (%)</th>
<th>Race, n (%)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>Male</td>
<td>Female</td>
<td>White</td>
<td>Black or African American</td>
</tr>
<tr>
<td>5 mg q.d. n=6</td>
<td>30.0±4.4</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>15 mg q.d. n=6</td>
<td>34.5±4.1</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>20 mg b.i.d. n=6</td>
<td>29.8±8.8</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>40 mg b.i.d. n=6</td>
<td>37.8±3.7</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>80 mg b.i.d. n=6</td>
<td>38.7±6.5</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>All LYS006 N=30</td>
<td>34.2±6.6</td>
<td>29 (96.7)</td>
<td>1 (3.3)</td>
<td>30 (100.0)</td>
</tr>
<tr>
<td>Placebo N=10</td>
<td>39.7±6.4</td>
<td>9 (90.0)</td>
<td>1 (10.0)</td>
<td>10 (100.0)</td>
</tr>
<tr>
<td>Total N=40</td>
<td>35.6±6.9</td>
<td>38 (95.0)</td>
<td>2 (5.0)</td>
<td>40 (100.0)</td>
</tr>
</tbody>
</table>

b.i.d., twice daily; BMI, body mass index; n, number of participants in individual cohort; N, number of participants; q.d., once daily; SAD, single ascending dose; SD, standard deviation.
**Supp. Table S9.** Summary statistics of skin and blister fluid PK parameter values of single ascending dose and multiple ascending dose

### Single Ascending Dose

<table>
<thead>
<tr>
<th>PK parameter (unit)</th>
<th>Profile Day</th>
<th>LYS006 30 mg q.d. n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (ng/mL)</td>
<td>2</td>
<td>4.7±0.9 (20.3)</td>
</tr>
<tr>
<td>Blister fluid/Plasma ratio</td>
<td>2</td>
<td>2.0±0.6 (32.8)</td>
</tr>
<tr>
<td>Blister fluid/Blood ratio</td>
<td>2</td>
<td>0.1±0.0 (26.4)</td>
</tr>
</tbody>
</table>

### Multiple ascending dose

<table>
<thead>
<tr>
<th>PK parameter (unit)</th>
<th>Profile Day</th>
<th>LYS006 15 mg q.d. n=6</th>
<th>LYS006 20 mg b.i.d. n=6</th>
<th>LYS006 40 mg b.i.d. n=6</th>
<th>LYS006 80 mg b.i.d. n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blister fluid concentration (ng/mL)</td>
<td>9</td>
<td>4.6±1.0 (21.1)</td>
<td>62.5±9.6 (15.4)</td>
<td>108.0±26.0 (24.1)</td>
<td>233.0±81.2 (34.8)</td>
</tr>
<tr>
<td>Blister fluid/Plasma ratio</td>
<td>9</td>
<td>1.7±0.2 (9.4)</td>
<td>2.1±0.7 (34.2)</td>
<td>2.6±2.1 (77.5)</td>
<td>2.0±0.9 (45.1)</td>
</tr>
<tr>
<td>Blister fluid/Blood ratio</td>
<td>9</td>
<td>0.1±0.0 (22.0)</td>
<td>1.1±0.2 (14.5)</td>
<td>1.6±0.5 (29.0)</td>
<td>2.0±0.6 (29.6)</td>
</tr>
<tr>
<td>Skin concentration (ng/g)</td>
<td>10</td>
<td>33.7±13.0 (38.5)</td>
<td>45.6±18.2 (40.0)</td>
<td>42.7±15.1 (35.3)</td>
<td>50.9±13.6 (26.7)</td>
</tr>
<tr>
<td>Skin/Plasma ratio</td>
<td>10</td>
<td>13.0±6.2 (47.9)</td>
<td>1.4±0.6 (38.9)</td>
<td>0.9±0.4 (45.6)</td>
<td>0.5±0.2 (43.6)</td>
</tr>
<tr>
<td>Skin/Blood ratio</td>
<td>10</td>
<td>0.9±0.2 (28.4)</td>
<td>0.8±0.3 (32.0)</td>
<td>0.6±0.2 (25.9)</td>
<td>0.4±0.1 (24.8)</td>
</tr>
</tbody>
</table>

Statistics are mean±SD (CV%), CV%=coefficient of variation (%)=SD/mean*100.
b.i.d., two times daily; n, number of participants in individual cohort; PK, pharmacokinetics; q.d., once daily; SD, standard deviation.
### Supp. Table S10. Summary statistics of urine PK parameter values of single ascending dose and multiple ascending dose

#### Single Ascending Dose

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Profile Day</th>
<th>LYS006 5 mg [n]</th>
<th>LYS006 10 mg [n]</th>
<th>LYS006 20 mg [n]</th>
<th>LYS006 30 mg [n]</th>
<th>LYS006 45 mg [n]</th>
<th>LYS006 70 mg b.i.d. [n]</th>
<th>LYS006 70 mg 100 mg b.i.d. [n]</th>
<th>LYS006 40 mg b.i.d. (limited hydration) [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount recovered (mg)</td>
<td>1</td>
<td>2.7±0.4 (13.0) [6]</td>
<td>5.8±0.7 (12.1) [6]</td>
<td>16.7±2.3 (13.7) [6]</td>
<td>23.3±2.6 (11.3) [6]</td>
<td>38.2±4.8 (12.5) [6]</td>
<td>51.3±11.9 (23.2) [5]</td>
<td>102.0±6.0 (5.9) [6]</td>
<td>190.0±9.0 (4.7) [6]</td>
</tr>
<tr>
<td>Fraction of dose excreted (%)</td>
<td>1</td>
<td>54.4±7.1 (13.0) [6]</td>
<td>57.5±6.9 (12.1) [6]</td>
<td>83.5±11.4 (13.7) [6]</td>
<td>77.5±8.8 (11.3) [6]</td>
<td>84.9±10.6 (12.5) [6]</td>
<td>73.3±17.0 (23.2) [5]</td>
<td>73.0±4.3 (5.9) [6]</td>
<td>95.0±4.5 (4.7) [6]</td>
</tr>
<tr>
<td>CLr_{\text{t}-6} (L/h)</td>
<td>1</td>
<td>15.7±2.4 (15.0) [6]</td>
<td>16.1±4.8 (29.5) [6]</td>
<td>15.3±3.3 (21.6) [6]</td>
<td>18.7±3.4 (18.4) [6]</td>
<td>18.2±6.0 (32.7) [6]</td>
<td>18.0±3.9 (21.8) [5]</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CLr_{\text{t}-12} (L/h)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CLr_{\text{t}-12} (L/h)</td>
<td>1</td>
<td>11.3±2.8 (24.9) [6]</td>
<td>13.7±2.1 (15.0) [6]</td>
<td>16.0±2.2 (13.7) [6]</td>
<td>13.9±2.7 (19.4) [6]</td>
<td>15.3±4.1 (26.6) [6]</td>
<td>17.8±2.1 (11.5) [5]</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CLr_{1.2-14} (L/h)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CLr_{1.2-14} (L/h)</td>
<td>1</td>
<td>6.5±1.4 (20.8) [6]</td>
<td>9.1±1.6 (17.3) [6]</td>
<td>10.9±4.0 (36.2) [6]</td>
<td>10.6±1.7 (15.7) [6]</td>
<td>9.4±3.1 (32.8) [6]</td>
<td>10.7±0.9 (8.0) [5]</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CLr_{1.4-16} (L/h)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>19.5±3.7 (19.2) [6]</td>
<td>18.7±2.8 (15.0) [6]</td>
</tr>
<tr>
<td>CLr_{1.6-18} (L/h)</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.5±1.2 (47.8) [6]</td>
<td>9.7±1.7 (18.0) [6]</td>
</tr>
<tr>
<td>CLr_{1.6-16} (L/h)</td>
<td>1</td>
<td>6.0±0.9 (15.1) [6]</td>
<td>9.6±2.1 (22.0) [6]</td>
<td>10.3±0.9 (9.0) [6]</td>
<td>10.6±1.5 (13.7) [6]</td>
<td>10.9±2.1 (19.3) [6]</td>
<td>14.0±3.1 (32.2) [6]</td>
<td>9.2±3.0 (53.7) [2]</td>
<td>13.1±7.0 (74.9) [2]</td>
</tr>
<tr>
<td>CLr_{1.6-18} (L/h)</td>
<td>1</td>
<td>4.9±0.9 (17.9) [6]</td>
<td>8.1±1.2 (15.0) [6]</td>
<td>8.0±1.3 (16.1) [6]</td>
<td>9.4±1.3 (13.2) [6]</td>
<td>9.8±2.3 (23.1) [6]</td>
<td>10.4±2.3 (21.5) [5]</td>
<td>9.3±4.3 (46.9) [4]</td>
<td>14.2±3.1 (22.0) [6]</td>
</tr>
</tbody>
</table>

#### Multiple Ascending Dose

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Profile Day</th>
<th>LYS006 5 mg q.d. n=6</th>
<th>LYS006 15 mg q.d. n=6</th>
<th>LYS006 20 mg b.i.d. n=6</th>
<th>LYS006 40 mg b.i.d. n=6</th>
<th>LYS006 80 mg b.i.d. n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount recovered (mg)</td>
<td>12</td>
<td>4.6±0.8 (18.0)</td>
<td>13.8±1.3 (9.6)</td>
<td>15.4±2.2 (14.3)</td>
<td>40.0±3.1 (7.8)</td>
<td>74.5±1.7 (15.7)</td>
</tr>
<tr>
<td>Fraction of dose excreted (%)</td>
<td>12</td>
<td>91.0±16.4 (18.0)</td>
<td>90.4±8.7 (9.6)</td>
<td>76.8±11.0 (14.3)</td>
<td>99.9±7.8 (7.8)</td>
<td>93.1±14.6 (15.7)</td>
</tr>
<tr>
<td>CLr_{\text{t}-2.25} (L/h)</td>
<td>12</td>
<td>22.5±3.7 (16.4)</td>
<td>21.7±3.6 (16.5)</td>
<td>17.6±7.0 (39.6)</td>
<td>27.0±3.1 (11.3)</td>
<td>19.3±2.5 (13.1)</td>
</tr>
<tr>
<td>CLr_{\text{t}-4.5} (L/h)</td>
<td>12</td>
<td>24.3±4.7 (19.3)</td>
<td>16.7±1.8 (11.0)</td>
<td>18.7±2.6 (13.9)</td>
<td>23.7±3.9 (16.4)</td>
<td>16.5±3.3 (19.8)</td>
</tr>
<tr>
<td>CLr_{\text{t}-6.5} (L/h)</td>
<td>12</td>
<td>14.3±7.1 (49.5)</td>
<td>14.7±7.8 (52.8)</td>
<td>14.6±2.4 (16.5)</td>
<td>18.7±1.7 (9.1)</td>
<td>13.2±4.2 (32.2)</td>
</tr>
<tr>
<td>CLr_{\text{t}-12.5} (L/h)</td>
<td>12</td>
<td>14.2±6.5 (45.9)</td>
<td>14.6±2.8 (18.8)</td>
<td>8.8±3.0 (34.3)</td>
<td>10.4±0.6 (5.4)</td>
<td>8.6±0.9 (10.7)</td>
</tr>
<tr>
<td>CLr_{1.2-25} (L/h)</td>
<td>12</td>
<td>9.2±2.4 (26.5)</td>
<td>10.1±2.1 (21.1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Statistics are mean±SD (CV%) [n], CV%=coefficient of variation (%)=SD/mean*100.

b.i.d., twice daily; CLr, renal clearance of the drug from plasma at steady state; h, hour; n, number of participants in individual cohort; PK, pharmacokinetics; q.d., once daily; SD, standard deviation.
**Supp. Table S11.** Elemental composition assignment of LYS006 and the metabolites based on high resolution mass spectrometry analysis

<table>
<thead>
<tr>
<th>Component</th>
<th>Elemental composition of [M+H]⁺</th>
<th>Theoretical [M+H]⁺ (m/z)</th>
<th>Measured [M+H]⁺ (m/z)</th>
<th>Difference between measured and theoretical [M+H]⁺ (mDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYS006</td>
<td>C₁₆H₁₅ClFN₆O₃</td>
<td>393.0878</td>
<td>393.0868</td>
<td>-1.0</td>
</tr>
<tr>
<td>M1</td>
<td>C₂₃H₂₈ClFN₇O₅</td>
<td>536.1824</td>
<td>536.1808</td>
<td>-1.6</td>
</tr>
<tr>
<td>M3 (MBR765)</td>
<td>C₁₄H₁₀ClFN₅O₃</td>
<td>350.0456</td>
<td>350.0444</td>
<td>-1.2</td>
</tr>
<tr>
<td>M4 (MBG479)</td>
<td>C₁₂H₈ClFN₅O₅</td>
<td>292.0401</td>
<td>292.0398</td>
<td>-0.3</td>
</tr>
<tr>
<td>M5</td>
<td>C₁₆H₁₄ClFN₅O₃</td>
<td>378.0769</td>
<td>378.0759</td>
<td>-1.0</td>
</tr>
<tr>
<td>M6</td>
<td>C₁₈H₁₇ClFN₆O₃</td>
<td>419.1035</td>
<td>419.1028</td>
<td>-0.7</td>
</tr>
<tr>
<td>M7</td>
<td>C₁₈H₁₇ClFN₆O₂</td>
<td>403.1086</td>
<td>403.1090</td>
<td>0.4</td>
</tr>
<tr>
<td>M9</td>
<td>C₂₃H₂₇ClFN₆O₅</td>
<td>521.1715</td>
<td>521.1729</td>
<td>1.4</td>
</tr>
</tbody>
</table>
**Figures**

**Supp. Fig. S1.** The calibration curves for the synthetic standards of the metabolites: Synthetic standards of M3 and M4 in human plasma (a, b); synthetic standards of M3 and M4 in human urine (c, d); [³H]-labeled M1 and M6 from rat, in human urine (e, f).
**Supp. Fig. S2.** The MS/MS spectra of LYS006 and the metabolites: LYS006 (a); M1 (b); M3 (c); M4 (d); M5 (e); M6 (f); M7 (g); M9 (h).

(a) MS/MS of LYS006 (m/z 393)
(b) MS/MS of M1 (m/z 536)
(c) MS/MS of M3 (m/z 350)
(d) MS/MS of M4 (m/z 292)
(e) MS/MS of M5 (m/z 378)
(f) MS/MS of M6 (m/z 419)
(g) MS/MS of M7 (m/z 403)
(h) MS/MS of M9 (m/z 521)
References
