### Safety Pharmacology

A total of 4 safety pharmacology studies were performed in accordance with Good Laboratory Practice (GLP) and guidelines of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

Tabulated results of safety pharmacology studies can be found in End-of-Text Table 1.2.

### Effects on CNS in Rats

PROJECT I was orally administered once to male rats at dose levels of 10, 100, and

1000 mg/kg (Study Project I-PT-0003). The observational parameters of the general activity and behavior were set by the modified Irwin's method. PROJECT I did not affect the general activity and behavior at doses up to 1000 mg/kg.

### Effects on hERG Current

The effects of PROJECT I on the hERG current were studied in hERG-transfected HEK293 cells by the whole-cell patch-clamp technique (Study Project I-PT-0001). The compensated suppression rates of PROJECT I at test concentrations of 1, 10, and 100 mcmol/L were −2.8%,

−0.1%, and 8.7%, respectively; a statistically significant difference from the control was noted at 100 mcmol/L.

### Effects on Action Potentials

The effects of PROJECT I on action potentials in isolated guinea-pig papillary muscles were studied by the microelectrode technique under a surface-superfusing condition (Study Project I-PT-0002). The test concentrations of PROJECT I were set at 1, 10, and 100 mcmol/L.

No effects on the action-potential parameters (action-potential duration at 30% repolarization [APD30], action-potential duration at 90% repolarization [APD90] and difference between APD30 and APD90 [APD30-90]), resting membrane potential, action-potential amplitude, or rate of maximum upstroke velocity (dV/dtmax) were noted at any test concentration.

### Effects on Central Nervous, Cardiovascular and Respiratory Systems in Dogs

PROJECT I was orally administered once to 4 male dogs implanted with transmitters of a telemetry system at dose levels of 10, 30, and 100 mg/kg under unanesthetized conditions (Study Project I-PT-0004). At the doses of 10 and 30 mg/kg, PROJECT I did not affect the general activity and behavior, body temperature, blood pressure, heart rate, ECG, respiration rate, blood gases, or blood electrolyte concentrations. At 100 mg/kg, vomiting was noted in

1 animal 43 min after administration, and slight increases in the heart rate between 1 and 4 h after administration (maximum percent change: +43%). All of the findings recovered thereafter.

### Pharmacodynamic Drug Interactions

No data for PROJECT I are available.

### Other Pharmacology Studies

No data for PROJECT I are available.

## Toxicology

A total of 16 toxicity studies were conducted in rats, dogs, mice and rabbits. All pivotal studies were performed in accordance with GLP and guidelines of ICH. An overview of toxicology studies of PROJECT I can be found in End-of-Text Tables 3.1, 3.2 and 3.3.

### Single-dose Oral Toxicity

Tabulated results of single-dose toxicity studies can be found in End-of-Text Table 3.4.

### Single-dose Oral Toxicity in Rats

PROJECT I was orally administered once at dose levels of 1000 and 2000 mg/kg to male and female rats (Study Project I-TX-0001). No death occurred in any groups. Moist fur around urethral orifice was observed in males and a female at 1000 mg/kg the day after administration and in females at 2000 mg/kg 6 h and the day after administration.

### Single-dose Oral Toxicity in Dogs

PROJECT I was orally administered once at dose levels of 200 and 500 mg/kg to male and female dogs (Study Project I-TX-0002). No death occurred in any groups. At 200 mg/kg, flush of auricle and conjunctiva was observed in the female from 2 h after administration and disappeared by 4 or 8 h after administration. At 500 mg/kg, flush of auricle and conjunctiva was observed in the male from 2 h after administration and disappeared by 24 h after administration. In the female flush of conjunctiva was observed from 2 h after administration and disappeared by 8 h after administration. In addition, a decrease in food consumption and no feces were observed on the administration day and its following day, respectively.

In the preliminary single-dose oral toxicokinetic study in dogs (Study Project I-TX-0016), 1 male died 2 days after a single oral administration of PROJECT I at 1000 mg/kg. In this male, flush (whole body, conjunctiva, oral mucosa and auricle), vomiting, decreased spontaneous motility and watery feces were observed after administration.

### Repeat-dose Oral Toxicity

Two preliminary 1-week oral toxicity studies in rats, 1 preliminary 1-week oral toxicity study in dogs and 4-week definitive oral toxicity studies in rats and dogs were conducted.

Tabulated results of repeat-dose toxicity studies can be found in End-of-Text Tables 3.5 and 3.6.

### 4-Week Repeat-dose Oral Toxicity in Rats

PROJECT I was orally administered once daily for 4 weeks at dose levels of 0, 10, 30, 100 and 1000 mg/kg/day to male and female rats (Study Project I-TX-0003). At 100 mg/kg/day, high serum total cholesterol was observed in both sexes, and low food consumption (only early stage), high urinary total sodium and chloride excretions, high neutrophil count, high thyroid weight, and hypertrophy of the centrilobular hepatocytes with high liver weight were observed in males. At 1000 mg/kg/day, low body weight and food consumption (only early stage), high urine volume and urinary total sodium and chloride excretions, low urine specific gravity, high mean corpuscular volume and reticulocyte ratio, high serum alanine aminotransferase (ALT) and total cholesterol, low serum sodium, hypertrophy of the centrilobular hepatocytes with high liver weight (revealed as increase in the smooth endoplasmic reticulum in the centrilobular hepatocytes by electron microscopy), and hypertrophy of the thyroid follicular cells with high thyroid weight were observed in both sexes. In males, low urinary pH, high hemoglobin concentration, hematocrit value, neutrophil count, and monocyte count, prolongation of activated partial thromboplastin time, high serum ALP, and high kidney weight were observed. The changes observed during the dosing period recovered or tended to recover during the 4-week recovery period. The NOAEL was 30 mg/kg/day for males and females.

### 4-Week Repeat-dose Oral Toxicity in Dogs

PROJECT I was orally administered once daily for 4 weeks at dose levels of 0, 3, 10, 30, and 100 mg/kg/day to male and female dogs (Study Project I-TX-0004). At 100 mg/kg/day, vomiting was observed in 1 male, and occult blood in urine was observed in 1 female. These changes recovered during the 4-week recovery period. The NOAEL was 30 mg/kg/day for males and females.

In the preliminary 1-week oral toxicity study in dogs (Study Project I-TX-0017), multifocal inflammation in the heart and ulcer in the kidney pelvis were observed in the male and female, respectively, at 100 mg/kg/day; however, these findings were not confirmed and no effect was seen on cardiac biomarkers in the above 4-week study.

### Genotoxicity

Tabulated results of genotoxicity studies can be found in End-of-Text Tables 3.7 and 3.8.

### In Vitro Reverse Mutation

A reverse mutation test was performed with *Salmonella typhimurium* (TA100, TA1535, TA98, and TA1537) and *Escherichia coli* (WP2*uvrA*), using the preincubation method with and without metabolic activation (Study Project I-TX-0005). Based on the results of the dose-finding test at 5, 15, 50, 150, 500, 1500, and 5000 mcg/plate, the main test was performed at 156, 313, 625, 1250, 2500, and 5000 mcg/plate. In comparison with the negative control, no 2-fold or greater increase in the number of revertant colonies was observed in any test strain with or without metabolic activation. It was concluded that PROJECT I has no potential to induce gene mutation in bacteria.

### In Vitro Chromosomal Aberration

A chromosomal aberration test was performed with cultured mammalian (Chinese hamster lung [CHL/IU]) cells in short-term treatments for 6 h with and without metabolic activation, and continuous treatment for 24 h without metabolic activation (Study Project I-TX-0006).

Chromosomal aberrations were analyzed at the following doses: 62.5, 125, 250, and

500 mcg/mL in short-term treatment with and without metabolic activation, and 50, 100, 150, 200, and 250 mcg/mL in continuous treatment for 24 h. The cell proliferation ratio determined from the population doubling showed dose-dependent decreases under all treatment conditions. No statistically significant increase in the number of cells with structural or numerical chromosomal aberrations was noted in any treatment group when compared with the negative control group. It was concluded that PROJECT I has no potential to induce chromosomal aberrations in CHL/IU cells.

### Micronucleus in Mice

PROJECT I was orally administered once daily for 2 days to male mice at dose levels of 500, 1000, and 2000 mg/kg/day (Study Project I-TX-0007). The number of micronucleated polychromatic erythrocytes (MNPCE) and the ratio of polychromatic erythrocytes (PCE) were investigated in femoral bone marrow at approximately 24 h after the final administration. No statistically significant increase in the number of MNPCE or decrease in the ratio of PCE was noted in any group when compared with the negative control group. It was concluded that PROJECT I did not induce micronuclei in mouse erythroblasts.

### Carcinogenicity

No studies have been performed with PROJECT I so far.

### Reproductive and Developmental Toxicity

Two definitive embryo-fetal development studies in rats and rabbits with dose-range finding studies were conducted. Tabulated results of reproductive and developmental toxicity can be found in End-of-Text Tables 3.10 and 3.12.

### Effects on Embryo-fetal Development

* + - * 1. **Effects on Embryo-fetal Development in Rats**

PROJECT I was orally administered once daily from day 7 to day 17 of gestation at dose levels of 30, 100, and 300 mg/kg/day to pregnant rats (Study Project I-TX-0011). In dams, low body weight gain and food consumption were noted during the early phase of the dosing period at 100 mg/kg/day and throughout the dosing period at 300 mg/kg/day. Loss of fur was observed in 3 dams from the latter phase of the dosing period at 300 mg/kg/day. In fetuses, low fetal body weight and placental weight were noted at 300 mg/kg/day. No test

article-related changes were noted in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, sex ratio, external, placental, skeletal, or visceral findings at up to 300 mg/kg/day. The NOAEL was 30 mg/kg/day for dams and 100 mg/kg/day for embryo-fetal development.

### Effects on Embryo-fetal Development in Rabbits

PROJECT I was orally administered once daily from day 6 to day 18 of gestation at dose levels of 10, 30, and 100 mg/kg/day to pregnant rats (Study Project I-TX-0012). In dams, no test article-related changes were observed up to 100 mg/kg/day. In fetuses, low fetal body weight was observed at 100 mg/kg/day. No test article-related changes were observed in the number of live fetuses, number of embryo-fetal deaths, postimplantation loss rate, sex ratio, placental weight, or external, placental, visceral or skeletal findings at up to 100 mg/kg/day. The NOAEL was 100 mg/kg/day for dams and 30 mg/kg/day for embryo-fetal development.

### Local Tolerance

No studies have been performed with PROJECT I so far.

### Other Toxicity Studies

Tabulated results of a phototoxicity study can be found in End-of-Text Table 3.16.

### 4.3.7.1 In Vitro Phototoxicity

A phototoxicity study was performed with cultured mammalian cells (Balb/c 3T3 cells) at 14.9, 23.8, 38.1, 61.0, 97.7, 156, 250, and 400 mcg/mL in the presence and absence of UV-A irradiation (Study Project I-TX-0013). The mean photo effect (MPE, actual value: −0.006) was less than 0.1. Therefore, PROJECT I was categorized as having no phototoxicity.

## 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

PROJECT I is a melatonin receptor agonist with high affinity for MT1 and MT2 melatonin receptors. PROJECT I induced a contraction of the isolated rat urethral strip. PROJECT I significantly increased urethral pressures at resting state and filling phase of the micturition cycle. PROJECT I increased LPP in rats at doses of 0.1 mg/kg id or more, indicating that PROJECT I has a potential to prevent leakage of urine under stress condition by increasing urethral pressure during storage. In contrast, PROJECT I did not increase the urethral pressure at voiding phase of the micturition cycle in rats up to 0.3 mg/kg id, and also did not affect the voiding efficiency or postvoid residual volume in rats up to 1 mg/kg id, suggesting that PROJECT I has a low risk for inducing voiding difficulty. Taken together, these results suggest that PROJECT I has a therapeutic potential in SUI.

In the safety pharmacology studies, PROJECT I did not have an effect on the CNS in rats at doses up to 1000 mg/kg. PROJECT I revealed weak hERG current inhibition (8.7%) at

100 mcmol/L. However, PROJECT I did not have an effect on action potentials in guinea pig papillary muscles up to 100 mcmol/L, nor an effect on ECG including QT interval corrected for heart rate in dogs at doses up to 100 mg/kg. Vomiting and increased heart rate were observed in dogs at doses of 100 mg/kg, however, they were transient and observed only at the high dose at which exposures were at large multiples of the pharmacologically effective concentration in rats.

PROJECT I absorption was extensive and rapid following oral administration, and PROJECT I was extensively distributed. Bioavailability slightly increased with dose in rats (68.6 to 109.0%) and dogs (45.8 to 71.8%). PROJECT I binds to plasma proteins (37.2 to 65.8%) in mice, rats, rabbits, dogs, and humans. Tissue distribution study showed negligible passage of PROJECT I and its metabolites across the blood-brain barrier, and elimination from almost all tissues was rapid.

The most prominent metabolite peak in human liver microsomes and hepatocytes was assumed to be AS3211400 (hydroxylated PROJECT I) and this was detected in all other species as a major peak. All other metabolite peaks in humans were also found in at least 1 other species, indicating that no human-specific metabolites were formed by liver microsomes or hepatocytes. PROJECT I and its metabolites were excreted by the renal (53.0%) and fecal (44.1%) routes in rats.

Based on in vitro nonclinical data, PROJECT I may inhibit the metabolism of substrates for CYP1A2 and the transport of substrates for BCRP. CYP1A2 is the principal isoform for the CYP-mediated metabolism of PROJECT I. Human plasma PROJECT I concentrations may be increased or decreased when coadministered with CYP1A2 inhibitors or inducers, respectively. PROJECT I is a substrate of BCRP.

The main targets for PROJECT I in the repeat-dose toxicity studies were the CNS, liver, thyroid gland, kidney, and hematopoietic system. Slightly high blood chemistry parameters (ALT, ALP, and cholesterol) and high liver weights with hypertrophy of hepatocyte were observed in rats. There was no hepatocyte damage in histopathology. Electron microscopy of the liver showed increases in smooth endoplasmic reticulum, suggesting metabolic enzyme induction in the liver. It is reported that hepatomegaly as a consequence of hepatocellular hypertrophy without histopathologically degenerative or necrotic changes is an adaptive nonadverse change and has little relevance to humans [Hall, 2012]. Increased thyroid gland weights with follicular cell hypertrophy are considered to be caused by increased clearance of thyroid hormones by metabolic enzyme induction in the liver. Changes in urinalysis in rats and dogs and high kidney weight in rats were not accompanied by histopathological changes in the kidney or urinary system. Changes in hematology in rats were not accompanied by histopathological changes in the hematopoietic system. All these findings were observed only at high dose(s) at which exposures were at large multiples of the pharmacologically effective concentration in rats and found to be reversible. Furthermore, they are considered to be monitorable in clinical trials.

Plasma exposure levels of PROJECT I in the repeat-dose toxicity studies are shown in [[Table 2](#_bookmark61)].

### Table 2 Plasma Exposure Levels of PROJECT I in Repeat-dose Toxicity Studies in Rats and Dogs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Species/ Study Duration** | **Doses (mg/kg/day)** | **Sex (M/F)** | **Steady-state Cmax (ng/mL)** | **Steady-state AUC24 (ng·h/mL)** |
| Rat, 4-week, po (Project I-TX-0003) | 10 | M | 2858.5 | 7195.1 |
| F | 2481.8 | 5375.2 |
| 30  (NOAEL for M/F) | M | 7022.7 | 31870.1 |
| F | 7049.7 | 21085.9 |
| 100 | M | 12844.9 | 133282.9 |
| F | 15491.0 | 141667.4 |
| 1000 | M | 33654.1 | 497636.1 |
| F | 36817.4 | 655811.3 |
| Dog, 4-week, po (Project I-TX-0004) | 3 | M | 985.2 | 1889.8 |
| F | 1168.0 | 2150.1 |
| 10 | M | 3869.3 | 15680.5 |
| F | 3115.7 | 11417.9 |
| 30  (NOAEL for M/F) | M | 5551.3 | 23926.8 |
| F | 7129.2 | 28066.0 |
| 100 | M | 12992.3 | 64034.9 |
| F | 14005.9 | 77944.4 |
| Rat, single, id  (Project I-PH-0009) | 0.1  (PAD) | F | 14.9 | - |

NOAEL: no observed adverse-effect level; id: intraduodenally; PAD: pharmacologically active dose. Mean values of Cmax and AUC24 are presented.

PROJECT I revealed no in vitro or in vivo genotoxic potential. PROJECT I revealed no teratogenic potential in rats or rabbits, although fetal growth retardation was observed at the highest dose tested. PROJECT I revealed no in vitro phototoxic potential.

Nonclinical findings and their relevance to human usage are summarized in [[Table 3](#_bookmark62)].

Overall, the nonclinical study package consisting of pharmacology, pharmacokinetics and toxicology supports clinical development of PROJECT I.

### Table 3 Summary of Nonclinical Findings and Their Relevance to Human Usage

|  |  |
| --- | --- |
| **Nonclinical Findings** | **Relevance to Human Usage** |
| <Pharmacological activity>  Agonistic action on MT1 and MT2 melatonin receptors | Potential for sleep disturbances and somnolence |
| <Nervous system from safety pharmacology>  Vomiting in dogs | Potential for vomiting and nausea |
| <Cardiovascular from safety pharmacology> Increase in heart rate in dogs | Potential for increase in heart rate |
| <Liver>  Slightly high ALT, ALP, and cholesterol  High liver weight, hepatocyte hypertrophy in rats (due to metabolic enzyme induction) | Little relevance to human usage |
| <Thyroid gland>  High thyroid weight, follicular cell hypertrophy in rats (due to metabolic enzyme induction in the liver) | Little relevance to human usage |
| <Kidney>  High urine volume, low urine specific gravity, high urinary total Na and Cl excretion, low urinary pH, high kidney weight in rats  Occult blood in urine in dogs | Potential for renal and urinary disorders |
| <Hematopoietic system>  High neutrophil count, high hemoglobin, hematocrit, MCV, reticulocyte ratio, and monocyte count | Potential for hematopoietic system disorders |
| <Drug interactions>  PROJECT I is a substrate and inhibitor of CYP1A2. Time-dependent inhibition of CYP1A2 was observed | CYP1A2 dependent drug interactions can be expected: strong CYP1A2 inhibitors (e.g., ciprofloxacin, fluvoxamine) may elevate PROJECT I plasma levels and PROJECT I may increase plasma levels of CYP1A2 substrates. Smoking behaviour (induction of CYP1A2) and caffeine intake (substrate of CYP1A2) (black/green tea, coffee, energy drinks) will be addressed. Volunteers should be non-smokers.  Potential for autoinhibition of PROJECT I metabolism |

ALP: alkaline phosphatase; ALT: alanine aminotransferase; CYP: cytochrome P450; MCV: mean cell volume.

#### List of References

Hall AP, Elcombe CR, Foster JR, Harada T, Kaufmann W, Knippel A, et al. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop. Toxicol Pathol. 2012;40:971-94.