Metabolism and Excretion of Asenapine in Healthy Male Subjects


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ABSTRACT:

The metabolism and excretion of asenapine [(3aRS,12bRS)-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3;6,7]-oxepino[4,5-c]pyrrole (2Z;2-butenedioate (1:1))] were studied after sublingual administration of [14C]asenapine to healthy male volunteers. Mean total excretion on the basis of the percent recovery of the total radioactive dose was ~90%, with ~50% appearing in urine and ~40% excreted in feces; asenapine itself was detected only in feces. Metabolic profiles were determined in plasma, urine, and feces using high-performance liquid chromatography with radioactivity detection. Approximately 50% of drug-related material in human plasma was identified or quantified. The remaining circulating radioactivity corresponded to at least 15 very polar, minor peaks (mostly phase II products). Overall, >70% of circulating radioactivity was associated with conjugated metabolites. Major metabolic routes were direct glucuronidation and N-demethylation. The principal circulating metabolite was asenapine N-c-glucuronide; other circulating metabolites were N-desmethylasenapine-N-carbamoyl-glucuronide, N-desmethylasenapine, and asenapine 11-O-sulfate. In addition to the parent compound, asenapine, the principal excretory metabolite was asenapine N-c-glucuronide. Other excretory metabolites were N-desmethylasenapine-N-carbamoyl-glucuronide, 11-hydroxyasenapine followed by conjugation, 10,11-dihydroxy-N-desmethylasenapine, 10,11-dihydroxyasenapine followed by conjugation (several combinations of these routes were found) and N-formylasenapine in combination with several hydroxylations, and most probably asenapine N-oxide in combination with 10,11-hydroxylations followed by conjugations. In conclusion, asenapine was extensively and rapidly metabolized, resulting in several regio-isomeric hydroxylated and conjugated metabolites.

Introduction

Asenapine [(3aRS,12bRS)-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3;6,7]-oxepino[4,5-c]pyrrole (2Z;2-butenedioate (1:1), Org 5222, Saphris) (Fig. 1) belongs to the group of dibenzoxepinopyrrolidine compounds and is available as a fast-dissolving tablet for sublingual administration.

Asenapine was approved for use in the United States for treatment of schizophrenia and for acute treatment as monotherapy or as an adjunct to lithium or valproate of manic or mixed episodes associated with bipolar I disorder in adults (Schering-Plough, 2010). Asenapine is also approved in Europe for the treatment of manic episodes in bipolar disorder (Merck & Co. wins European Union agency support for antipsychotic, 2010, http://www.reuters.com/article/usUSLDE65O0RE20100625). Asenapine has a unique receptor-binding profile, with potent multireceptor antagonism for a combination of serotonin, dopamine, noradrenaline, and histamine receptors, with a higher absolute affinity than currently available antipsychotics for a subset of therapeutically relevant serotonergic (5-HT2A, 2B, 2C, 6, and 7), noradrenergic (α1 and α2), and dopaminergic (D3 and D4) receptors and no appreciable activity at muscarinic cholinergic receptors (Shahid et al., 2009).

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ABBREVIATIONS: LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; SPE, solid-phase extraction; LC, liquid chromatography; MS/MS, tandem mass spectrometry; MS, mass spectrometry; ES, electrospray; a.o., among others; MIM, monoisotopic molecular mass; TOCSY, total correlation spectroscopy; 2D, two-dimensional; SK&F 86466, 6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine.
Materials and Methods

Study Execution and Dose Preparation and Administration. The clinical part of this study was performed in the clinical research center of PRA (Zuidlaren, The Netherlands). Six healthy male volunteers (ages 21–54 years; body mass index, 20.5–28.1 kg/m²) received sublingual unlabeled asenapine according to the schedule in Table 1, which has previously been demonstrated to be well tolerated (Dogterom et al., 2009). It was anticipated that healthy subjects would not tolerate an immediate single dose of 10 mg, and, therefore, it was decided to gradually increase the dose by titrating to 10 mg and administer the dose until steady state, which was expected after 6 days of dosing, before administration of the radioactive dose. All doses were given twice a day except for the radioactive dose, which was administered as a single sublingual dose on the morning of day 10. Subjects were hospitalized from day 2 until 10 days after the final radioactive dose. Four subjects completed the study; two subjects withdrew before receiving the radioactive dose.

The study was approved by the appropriate medical ethics committee and conducted in full compliance with the Declaration of Helsinki and the principles of Good Clinical Practice. Laboratory assessments were performed in full compliance with Good Laboratory Practice. All study participants signed an informed consent form before screening evaluations.

The radiolabeled asenapine sublingual tablets were prepared by dispensing [14C]-labeled asenapine in ethanol on the tablets. Ethanol was removed under reduced pressure at room temperature. The average radioactivity content of the tablets as determined in six tablets was 2.07 MBq (relative S.D. = 2.1%), and no radiochemical degradation was found; the radiochemical purity was >97%.

Chemicals and Reference Compounds. [14C]-Asenapine (label at C12b, N-desmethylasenapine, and asenapine N-oxide in human plasma samples was performed by a validated assay using an internal standard method after SPE at the Department of Bioanalytics, Essex Pharma Development GmbH (Waltrop, Germany). For the main metabolite, asenapine N°-glucuronide, no standard was available at the time of bioanalysis.

The extracts were quantified by LC-MS using ES ionization in multireaction monitoring mode (data on file, 2008). The Watson Drug Metabolism Laboratory Information Management System (version 6.3.0.03) was used for all calculations. Calibration curves for asenapine, N-desmethylasenapine, and asenapine N-oxide were constructed using linear weighted (1/x²) regression.

Pharmacokinetics. All calculations were performed using the SAS system for Windows (version 8.2 running under Windows XP at the Department of Clinical Pharmacology and Kinetics, Merck Sharp and Dohme). Plasma concentration-time curves of asenapine, asenapine N-oxide, N-desmethylasenapine, and total radioactivity ([14C] expressed in mass equivalents of asenapine) were constructed for each of the four individuals completing the study. Pharmacokinetic parameters were calculated from the plasma concentration-time data, where feasible.

Metabolite Profiling, Isolation, and Identification. Individual and pooled plasma samples were used for metabolite profiling and identification. Plasma samples containing sufficient radioactivity were analyzed individually per subject and per time point. In addition, plasma samples were pooled across subjects, per time point or per time interval. Peaks were identified by retention time comparison with authentic reference compounds and LC-MS analysis, where feasible.

Individual urine and fecal samples containing more than 5% of the administered radioactivity were selected for metabolite profiling. These urine and fecal samples were pooled per matrix and per individual in such a way that quantification of metabolites reflects the situation in the excreta.

Sample Treatment. Acidified (0.2% HCl, v/v %) plasma and urine samples were applied to preconditioned Oasis HLB SPE columns (Waters, Milford, MA). The columns were washed with Milli-Q water and eluted with acetonitrile-0.01 M NH₄OH, pH 4.2 (7.5, v/v %). The eluate samples were concentrated under a gentle stream of nitrogen, centrifuged (for urine samples), and analyzed by HPLC. The recovery of SPE was determined by LSC and was ≥81% for plasma samples. For urine samples, the mean recovery was approximately 89%.

Fecal samples were extracted with 2 volumes of acetonitrile-methanol (3:1, v/v %) and 1% acetic acid [feces homogenate/extraction solution ratio (w/v, 1:2%)]. In addition, the fecal samples were extracted with acetonitrile-methanol (3:1, v/v %) [feces homogenate/extraction solution ratio (w/v, 1:2%)]. The extraction recovery was determined by extraction by LSC. The extracts containing >5% of the radioactivity were pooled and concentrated under a gentle stream of nitrogen. The concentrated extracts were analyzed by HPLC. The mean extraction recovery for feces was 74%.

Metabolite Profiling. The metabolic profiling of asenapine in pooled plasma, urine, and fecal samples was performed on an HPI-100 liquid chromatograph (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) equipped with a diode array detector using a Bondapak C18 column (internal diameter, 7.8 mm; internal length, 30 cm; particle size, 10 μm) and a gradient of ammonium acetate buffer (0.1 M, pH 4.2) (solvent A) and methanol-acetonitrile (1:3, v/v %) (solvent B) at 50°C. Elution started with 3 min isocratic at 10% solvent B followed by a linear gradient of 10 to 40% solvent

Sample Collection. Blood samples for determination of pharmacokinetics of asenapine and its N-desmethyl and N-oxide metabolite and of the concentration of total radioactivity in plasma were collected from day 10 onward at 0 (predose) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, and 72 h postdose using heparin as an anticoagulant. In addition, blood samples for metabolite profiling were collected on day 1 at 0 (predose; i.e., just before the first dose of asenapine) and on day 10 at 1, 1.5, 2, 4, 8, 12, and 24 h postdose, using EDTA as an anticoagulant. Plasma was prepared by centrifugation. Urine and feces were collected up to 264 h postdose. Plasma, urine, and fecal samples were stored at -20°C until analysis.

Determination of Radioactivity in Plasma, Urine, and Feces. The amount of radioactivity in plasma and urine was determined by LSC. Feces were homogenized with approximately 2 volumes of Milli-Q water, after which the amount of radioactivity in feces homogenates was determined by combustion in a sample oxidizer followed by LSC.

Bioanalysis. Bioanalysis of asenapine, N-desmethylasenapine, and asenapine N-oxide in human plasma samples was performed by a validated assay using an internal standard method after SPE at the Department of Bioanalytics, Essex Pharma Development GmbH (Waltrop, Germany). For the main metabolite, asenapine N°-glucuronide, no standard was available at the time of bioanalysis.

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Reference compounds used for identification of metabolites isolated from plasma, urine, and feces after sublingual dosing of unlabeled asenapine followed by a single sublingual dose of [14C]asenapine

<table>
<thead>
<tr>
<th>Org Code</th>
<th>Short Metabolite Name</th>
<th>Chemical Name</th>
</tr>
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<tbody>
<tr>
<td>Org 5222</td>
<td>Aseinapine</td>
<td>(3aRS,12bRS)-5-Chloro-2-methyl-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole (2Z,2)-2-butenedioate (1:1)</td>
</tr>
<tr>
<td>Org 30526</td>
<td>N-Desmethylasenapine</td>
<td>trans-5-Chloro-2-H,2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole maleate</td>
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<tr>
<td>Org 31437</td>
<td>Aseinapine N-oxide</td>
<td>trans-5-Chloro-2,3,3,a,12b-tetrahydro-2-methyl-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole 2-oxide</td>
</tr>
<tr>
<td>Org 207652-0</td>
<td>N-Formylasenapine</td>
<td>trans-5-Chloro-2-formyl-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
</tr>
<tr>
<td>Org 213772-0</td>
<td>11-Hydroxyasenapine</td>
<td>trans-5-Chloro-11-hydroxy-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole hydrochloride</td>
</tr>
<tr>
<td>Org 21393-1</td>
<td>11-Hydroxy-N- desmethylasenapine</td>
<td>trans-5-Chloro-11-hydroxy-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
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<tr>
<td>Org 214025-0</td>
<td>Aseinapine 11-O-sulfate</td>
<td>trans-5-Chloro-11-sulfooxy-2-methyl-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
</tr>
<tr>
<td>Org 216761-0</td>
<td>N'-glucuronide</td>
<td>trans-5-Chloro-2-β-O-glucopyranuronosyl-2-methyl-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrolin</td>
</tr>
<tr>
<td>Org 220473-0</td>
<td>7-Hydroxyasenapine</td>
<td>trans-5-Chloro-7-hydroxy-2-methyl-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
</tr>
<tr>
<td>Org 213979-0</td>
<td>11-Hydroxy-N-formylasenapine</td>
<td>trans-5-Chloro-2-formyl-11-hydroxy-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
</tr>
<tr>
<td>Org 237498-0</td>
<td>11-Methoxyasenapine</td>
<td>trans-5-Chloro-2-methyl-11-methoxy-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole</td>
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<tr>
<td>Org 225789-0</td>
<td>11-Hydroxyasenapine N-oxide</td>
<td>trans-5-Chloro-2,3,3,a,12b-tetrahydro-11-hydroxy-2-methyl-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole 2-oxide</td>
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<tr>
<td>Org 191475-0</td>
<td>N-Desmethylasenapine-N-carbamoylglucuronide</td>
<td>1-((3aR,12bR)-Rel-5-chloro-2,3,3,a,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole-2-carboxylate)-α-glucopyranuronic acid</td>
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</table>

B in 17 min, 40 to 90% solvent B in 30 min, and 90 to 95% solvent B in 1 min. This solvent composition was held during at least 3 min and maximally 8 min before returning to starting conditions. The flow rate was 2.0 ml/min.

Several reference compounds, monitored by UV detection (270 nm), were analyzed by HPLC, before or after analysis of the study samples. Radioactivity in the HPLC effluent was determined online using a flow-through detector (Flo-One Beta model A500TR; PerkinElmer Life and Analytical Sciences, Zaventem, Belgium) or offline by the collection of fractions followed by solid scintillation counting.

**Isolation of Metabolites.** Pooled urine samples were filtered, acidified, and extracted by SPE. The eluate samples were concentrated and injected on HPLC, after which fractions were collected. Fractions constituting a peak of radioactivity (U1–U10) were pooled and concentrated under a gentle stream of nitrogen gas. The isolated metabolite fractions were purified by SPE using Oasis HLB columns with acetonitrile-0.01 M NH₄OAc, pH 4.2 (7:3, v/v %) and methanol-0.01% trifluoracetic acid, respectively.

Fecal samples (homogenates) were pooled and extracted. The extracts were pooled, concentrated, and injected onto the HPLC system, after which fractions were collected. Fractions constituting a pool were pooled (F1–F11), concentrated, and further purified by acetonitrile-methanol-acetic acid extraction followed by extraction with diethyl ether.

On analysis, some of the fractions consisted of more metabolites, and attempts were made to purify these fractions using a variety of SPEs, liquid-liquid extractions, HPLC columns, elution profiles, and flow rates.

**Hydrolysis of Urinary Metabolites.** Metabolites U1 and U2/3, isolated from urine, were incubated in a solution containing sulfatase and β-glucuronidase to identify the phase II (conjugated) metabolites. These metabolites were dried under a stream of nitrogen, dissolved in a drop of methanol-0.01% trifluoracetic acid, and diluted with 0.1 M ammonium acetate buffer (pH 6). Subsequently, these metabolites were incubated for 18 h at 37°C in the presence of sulfatase and β-glucuronidase. In addition, incubation without sulfatase and β-glucuronidase was performed as a control. Incubation mixtures were pretreated by SPE and subjected to HPLC analysis.

**Identification of Metabolites.** A number of metabolites found in plasma, urine, and feces were tentatively identified by HPLC retention time comparison with authentic reference compounds and a previously isolated and identified metabolite (N-desmethylasenapine-N-carbamoylglucuronide) (von dem Wildenberg et al., 1990). Confirmation of identities of the metabolites was obtained by LC-MS/MS and, where feasible, with NMR or enzymatic deconjugation.

**NMR Spectroscopy.** 1H Spectra were recorded at 600 MHz on a Bruker Avance 600 spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) under standard conditions. The samples were dissolved in CD₃OD. The CD_OD was used as a reference and set to 3.30 ppm. The chemical shifts are given in parts per million.

**Mass Spectrometry.** ES mass spectra and product ion scans were recorded in positive and negative ion mode using the ionspray interface on the ABI MDS Sciex QStar Pulsar hybrid QqTOF mass spectrometer (MDS Sciex, Concord, ON, Canada) or the ES interface on the Bruker Esquire 3000 Plus quadrupole ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). A sample introduction was performed by HPLC, using different HPLC methods. The flow was split before it entered the mass spectrometer. The PE Sciex QStar Pulsar mass spectrometer was operated at a positive ionspray voltage of approximately 5500 V. Although the mass resolution of the QStar in MS mode was >6500, only nominal masses are reported. Mass spectra were recorded from approximately m/z 150 to m/z 1000 or 1200. The Bruker Esquire 3000 Plus was operated in positive and negative ion mode. The mass spectra were recorded from m/z 150 to 750.

**Results**

**Plasma Concentration of Asenapine and Total Radioactivity.** Mean plasma concentrations of asenapine plus metabolites expressed in nanogram equivalents per milliliter of plasma were calculated from the total radioactivity concentrations in plasma samples and the specific activity of the [14C]-labeled formulated sublingual dose. The asenapine concentration in plasma was determined by bioanalysis.

Plasma concentrations of total radioactivity greatly exceeded those of asenapine from the first time point (0.5 h after radioactive dosing) onward (Fig. 2). The peak concentration of 14C radioactivity was reached 4 h after dosing, later than that for asenapine (0.75 h). Half-lives (mean ± S.D.) of total radioactivity (39.3 ± 7.6 h) and asenapine (27.5 ± 5.0 h) in plasma were in the same range or slightly longer for total radioactivity, suggesting that the metabolites of asenapine have a comparable or slightly longer half-life. The metabolites analyzed (N-desmethylasenapine and asenapine N-oxide) constituted only a small fraction of the total of asenapine metabolites in plasma.

**Excretion of Radioactivity in Urine and Feces.** After sublingual administration of [14C]asenapine to healthy male volunteers under steady-state conditions, radioactivity was excreted via urine and feces as shown in Fig. 3. The cumulative excretion of radioactivity via urine was ~50%, whereas the cumulative excretion in feces was ~40%. The total recovery of radioactivity was ~90%; more than 80% of the radioactive dose was excreted within 96 h.

**Metabolite Profiling and Characterization.** Radioactive peaks in the HPLC profiles were numbered per matrix on the basis of retention time. Asenapine was extensively and rapidly metabolized and resulted in several regio-isomeric hydroxylated and conjugated metabolites. Nearly 50% of drug-related material in human plasma was identified or quantified. The remaining radioactivity (~50%) corresponds to different very polar peaks, none of which represent >6% of the

**TABLE 2**

Reference compounds used for identification of metabolites isolated from plasma, urine, and feces after sublingual dosing of unlabeled asenapine followed by a single sublingual dose of [14C]asenapine
plasma radiocarbon profile. Metabolites eluting in this region have been tentatively identified and correspond mostly to phase II (sulfate, glucuronide, and methylated) products. Overall, >70% of circulating radioactivity was associated with conjugated metabolites. In addition, a significant percentage (~71%) of the excreted radioactivity was characterized. The most important MS/MS fragments and related metabolite structures of the identified metabolites in plasma, urine, and feces are given in Table 3. The metabolic routes of asenapine are shown in Fig. 4.

**Plasma.** The metabolite profiles of pooled plasma samples (1 and 1.5–12 h) consisted of at least six peaks (P1–P6). Peak P2/3 was the major metabolite in both pooled plasma samples. In addition, peak P6 was a major metabolite in the 1-h pooled plasma sample. The metabolite profile of the 1.5- to 12-h pooled plasma sample is given in Fig. 5.

**P1: asenapine 11-O-sulfate.** The HPLC retention time and LC-ES-MS spectra (m/z 382/384) of metabolite P1 eluting at 22.4 min were identical to those of the reference compound Org 214025-0 [asenapine 11-O-sulfate, trans-5-chloro-11-sulfooxy-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3:6,7]-oxepino[4,5-c]pyrrole]. On the basis of LC-MS data, this peak also contained some other hydroxylated and conjugated metabolites.

**P2/3: asenapine N'-glucuronide.** Peak P2/3 eluting at 25 min was a major metabolite in the pooled plasma samples and coeluted with reference compound Org 216761-0 (trans-5-chloro-2(β-D-glucopyranuronosyl)-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3:6,7]-oxepino[4,5-c]pyrrolium, asenapine N'-glucuronide). The fragmentation patterns of the reference compound and P2/3 both resulted in the main ions m/z 462/464, which corresponded to the cation of asenapine N'-glucuronide. In addition, the product ion spectrum (MS/MS) of m/z 462 (286, 159, and 131, a.o., loss of 176 Da, characteristic for the glucuronide structure) was in agreement with asenapine N'-glucuronide. The fraction consisted of diastereoisomeric forms that were partly separated under the HPLC conditions applied. For these reasons, peak P2/3 was identified as the asenapine N'-glucuronide.

**P4: N-desmethylasenapine-N-carbamoyl-glucuronide.** Peak P4 eluting at 28.7 min coeluted with reference compound Org 191475-0 (N-desmethylasenapine-N-carbamoylglucuronide). LC-ES-MS analyses of this fraction resulted in the main ions m/z 492/494, which
<table>
<thead>
<tr>
<th>Metabolite Identification</th>
<th>Proposed Structure</th>
<th>Precursor Ion</th>
<th>Product Ions (Positive Ion Electrospray)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 U6/7A</td>
<td>Asenapine 1-O-sulfate</td>
<td>382 (QqTOF) 302 (loss of SO$_3$)</td>
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</tr>
<tr>
<td>P2/3 U8/9A + B</td>
<td>Asenapine N$^+$-glucuronide</td>
<td>462 (QqTOF) 286, 159, 131</td>
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<tr>
<td>P4 U10</td>
<td>N-Desmethylasenapine-N-carbamoylglucuronide</td>
<td>492 (QqTOF) 316, 272</td>
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<tr>
<td>P5 F8</td>
<td>N-Desmethylasenapine</td>
<td>272 (QqTOF) 229, 215, 201, 166</td>
<td></td>
</tr>
<tr>
<td>P6 F9</td>
<td>Asenapine</td>
<td>286 (QqTOF) 229, 215, 201, 166</td>
<td></td>
</tr>
</tbody>
</table>
corresponded to the [M + H]\(^+\) of N-desmethylasenapine-N-carbamoylglucuronide. In addition, the product ion spectrum (MS/MS) of \(m/z\) 492 (316 and 272, a.o., loss of 176 Da, glucuronide) was in agreement with this reference.

**P5: N-desmethylasenapine.** LC-ES-MS analyses of peak P5 eluting at 29.7 min resulted in the main ions \(m/z\) 272/274, which corresponded to the [M + H]\(^+\) of the reference compound Org 30526 (N-desmethylasenapine; \(N\)-desmethylasenapine; \(trans\)-5-chloro-2-\(H\)-2,3,3\(_a\),12b-tetrahydro-1\(H\)-dibenzo[2,3:6,7]-oxepin[4,5-c]pyrrole maleate). In addition, the product ion spectrum (MS/MS) of \(m/z\) 272 (a.o., loss of 43 Da, \(C_3H_7N\), characteristic for the \(N\)-desmethyl structure) and the retention time of this compound was in agreement with this reference.

**P6: asenapine.** Peak P6 eluting at 30.2 min was a major peak of moiety in the 1-h pooled plasma sample and coeluted with reference compound Org 5222 (asenapine). The fragmentation patterns of P6 resulted in the main ions \(m/z\) 286/288, which corresponded to asenapine. In addition, the product ion spectrum (MS/MS) of \(m/z\) 286 (a.o., loss of 57 Da, \(C_4H_7N\), characteristic for the \(N\)-methyl structure) and the retention time of this compound was in agreement with the reference. In addition, \(m/z\) 302/304 was observed. Additional peaks found in the pooled plasma samples could not be identified, with no chlorine pattern observed in the MS analyses.

**Urine.** The metabolite profiles of urine samples consisted of at least 10 different peaks (U1–U10). Peak U8/9 was the major metabolite, representing 10 to 21% of the radioactive dose. In addition, peaks U2/3, U4/5, U6/7, and U10 represented >5% of the administered radioactive dose at least in one of the pooled urine samples. The other peaks (including peak U1) were minor peaks (<3% of the administered radioactive dose) in all urine samples. A representative metabolite profile is shown in Fig. 6.

**U1: mixture of the methoxy, the glucuronide of 10,11-dihydroxy-N-desmethylasenapine, and the glucuronide of 10,11-dihydroxy-N-desmethylasenapine.** LC-ES-MS analyses of peak U1 resulted in two fractions (U1A and U1B) in which the MIMs were 493 and 479. The
product ion spectrum (MS/MS) (Table 3) of MIM 493 in sample U1A was slightly different from that of the other sample U1B (most probably the position of the methoxy and glucuronide interchanged). Both samples showed a loss of 176 Da, characteristic for a glucuronide, followed by, a.o., a loss of 43 Da (C₂H₅N) as in the N-desmethyl. This fraction was identified as the methoxy and glucuronide conjugate of 10,11-dihydroxy-N-desmethylasenapine, in which the position of the methoxy and glucuronide is 10,11 and the reverse.

Analog to the fraction with MIM 493 and on the basis of the mass difference of 14 Da between the fractions with MIMs of 479 and 493, the fraction with MIM of 479 is tentatively identified as the glucuronide of the 10,11-dihydroxy-N-desmethylasenapine. Enzymatic hydrolysis of U1A/H11001/B released ES-MS spectra containing MIM 317, corresponding with the O-methylated-10,11-dihydroxy-N-desmethylasenapine. The product ion spectrum (MS/MS) of the protonated molecule showed, a.o., the loss of 43 Da (C₂H₅N) toward m/z 275; the MS/MS/MS product ion spectrum of m/z 275 showed losses of both 15 Da (CH₃) and 32 Da (CH₃OH). Furthermore, after H–D exchange in D₂O/CH₃COOD, two exchangeable protons were found (MIM 319), in agreement with the monohydroxy, monomethoxy, N-desmethyl structure proposed.

Fig. 4. Proposed metabolic routes of asenapine. * very low levels of asenapine N-oxide are detected by LC-MS analysis in plasma. In urine this metabolite was only found in combination with other metabolic reactions. **, only found in combination with other metabolic reactions. *** asenapine is a racemic mixture.
The metabolites of asenapine include sulfate and glucuronide derivatives. The sulfate isomer is more likely to be 10,11 or the reverse, and the glucuronide of 11-hydroxy-asenapine may be present. Further evidence for the proposed structures was obtained from the ES-MS spectra of the hydrolyzed U2/3A fraction containing MIMs 317, 287, and 301, which were in agreement with the hydroxy-mono, monomethoxy, and N-glucuronide structure in peak U1, 11-hydroxy-N-desmethylasenapine, and desmethylasenapine.

Based on these results, peak U2/3 was identified as a mixture of trans-5-chloro-10-methoxy-11-sulfoxy-2,3,3a,12b-tetrahydro-1H-dibenzo[c,2,3:6,7]-oxepino[4,5-c]pyrrole-11-yl β-d-glucopyranosiduronic acid plus some other unidentified conjugated metabolites (fractions with MIMs 383, 493, and 507).

**U4/5**: mixture of conjugated metabolites. LC-ES-MS analyses of peak U4/5 resulted in two fractions (U4/5A and U4/5B) containing at least five MIM values, namely 397, 411, 507, 477, and 427. No structural proposals could be given for these fractions, but on the basis of fragmentation patterns, it can be concluded that peak U4/5 consisted of several conjugated metabolites (sulfates and glucuronides).

**U6/7**: mixture of asenapine 11-O-sulfate, the monohydroxylated, mono-O-sulfated asenapine N-oxide, and the mono-O-sulfated asenapine N-oxide. LC-ES-MS analyses of peak U6/7 resulted in five fractions coded U6/7A to U6/7E. The major signals in the 1H NMR spectrum of fraction U6/7A were comparable to the signals for the reference compound asenapine 11-O-sulfate. U6/7A resulted in two MIM values, namely 381 and 517 (very small peak). Based on the NMR, MS, and MS/MS data and retention time comparison, the fraction with MIM 381 was identified as the asenapine 11-O-sulfate. U6/7B showed an MIM value of 413. On the basis of the loss of 80 Da and the presence of m/z 57 in the product ion spectrum of the protonated molecule, it can be concluded that MIM 413 contained a sulfate and is most probably an N-oxide of monohydroxylated, mono-O-sulfated asenapine. U6/7C showed two MIM values, namely 397 and 412. On the basis of the loss of 80 Da and presence of m/z 57, it can be concluded that MIM 397 contained a sulfate and is most probably an N-oxide of mono-O-sulfated asenapine. No structure proposal could be given for MIM 412, but on the basis of the fragmentation (a loss of 176 Da) pattern, it can be concluded that this fraction contained a glucuronide. U6/7D showed three MIM values, namely 301, 491, and 499. MS and MS/MS data showed that MIM 301 was in agreement with 11-hydroxyasenapine. Because the retention time of this compound (based on comparison with the 11-hydroxyasenapine reference) differs from the retention time of U6/7, this monohydroxylated metabolite probably is an isomer. Based on the loss of 176 Da in the product ion spectrum of the protonated molecules, it can be concluded that MIM 491 and 499 both contained a glucuronide. For MIM 499, three times the H2O loss was observed in the MS/MS analysis. For MIM 491, no further structural evidence was obtained. U6/7E resulted in three MIM values, namely 491, 477, and 301. The signal/noise ratio for MIM 301 was very low. No structure proposal could be given for these fractions, but on the basis of fragmentation (loss of 176 Da) for MIM 491 and 477, it can be concluded that these fractions both contained a glucuronide.

**U8/9**: asenapine N'-glucuronide. Peak U8/9 was the major metabolite in urine, coeluted with the reference compound Org 216761-0 (asenapine N+–glucuronide). The fragmentation patterns of the reference compound and U8/9 resulted in the main ions m/z 462/464.
which corresponded to the cation of the asenapine $N^\ominus$-glucuronide. In
addition, the product ion spectrum (MS/MS) of $m/z$ 462 (a.o., loss of
176 Da, characteristic for the glucuronide structure) and the $^1$H NMR
spectra were in agreement with the asenapine $N^\ominus$-glucuronide.

$U10$: $N$-desmethylasenapine-$N$-carbamoyl-glucuronide. The $^1$H
NMR spectrum of peak $U10$ was in agreement with NMR data for the
previously isolated and identified metabolite Org 191475-0 ($N$-
desmethylasenapine-$N$-carbamoylglucuronide) (von dem Wildenberg et
al., 1990). In addition, the MS, MS/MS, and retention time were in
agreement with those of this reference compound.

**Feces.** The peaks present in fecal samples consisted of at least 11
different peaks (F1–F11), of which asenapine (F9, representing
5–16% of the radioactive dose) was the major peak. In addition, peak
F1/2 (representing 6–10% of the radioactive dose) and two minor
peaks (F10 and F11) were isolated and identified. Peaks F3–F7 could
not be further characterized. Peak F8 coeluted with $N$-desmethyla-
senapine but was not further characterized. A representative metabo-
lite profile is shown in Fig. 7. (feces, 48–72 h, subject 1).

**F1/2:** 10,11-dihydroxy-$N$-desmethylasenapine and 10,11-dihy-
droxyasenapine. LC analyses of peak F1/2 resulted in two fractions
coded F1/2A and F1/2B. The $^1$H NMR and TOCSY (2D NMR)
spectra of peak F1/2A showed many unknown, unrelated aliphatic and
some small broader aromatic impurities. Some sharper aromatic signals
were present. The LC-ES-MS spectrum of fraction F1/2A contained
the loss of 43 Da, C$_2$H$_5$N, in the product ion scan of the protonated
molecule), the compound was most probably asenapine with two hydroxyl
groups. On the basis of the chemical shift and the pattern of the sharp aromatic protons
and the absence of the signal at 2.95 ppm (compared with
F1/2B) in the NMR spectra and MS results, the structure of F1/2A is
most probably the trans-5-chloro-10,11-dihydroxy-2,3,3$a$,12b-tetra-
hydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole.

The $^1$H NMR and TOCSY (2D NMR) spectra of peak F1/2B showed
many unknown, unrelated aliphatic and some small
broader aromatic impurities. Some sharper aromatic signals (two
singlets at 6.41 and 6.57 ppm H9 and H12, doublet at 7.06 ppm H7,
doublet at 7.08 ppm H4, and doublet doublet at 7.15 ppm H6) and
a singlet at 2.95 ppm were present. LC-ES-MS analyses of this fraction resulted in the main ions $m/z$ 318/320 (M $+ \text{H}^+$). On the basis of the MS-MS results (a.o. an observed loss of 57 Da, C$_4$H$_7$N,
in the product ion scan of the protonated molecule), the compound is most probably asenapine with two hydroxyl groups. On the basis
of the chemical shift and the pattern of the sharp aromatic protons
and the chemical shift of the signal at 2.95 ppm (N–CH$_3$) in the
NMR spectra and MS results, the structure of F1/2B is most probably
trans-5-chloro-10,11-dihydroxy-2-methyl-2,3,3$a$,12b-tetrahydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole.

**F9:** asenapine. The $^1$H NMR and TOCSY (2D-NMR) spectra of
peak F9 were compared with those of a primary reference standard of
asenapine. The asenapine-related entity in the F9 fraction was in
agreement with asenapine. In addition, some unknown, unrelated
aromatic and many unknown, unrelated aliphatic compounds were
present. The LC-ES-MS spectrum of fraction F9 resulted in the main
ions $m/z$ 286/288, which corresponded to the [M $+ \text{H}^+$] of
asenapine. The product ion spectrum (MS/MS) of $m/z$ 286 was also in agreement with that of asenapine.

**F10:** 11-hydroxy-$N$-formylasenapine. The $^1$H NMR spectra of peak
F10 were in agreement with those of Org 213979-0 (11-hydroxy-$N$-
formylasenapine, trans-5-chloro-2-formyl-11-hydroxy-2,3,3$a$,12b-tetra-
hydro-1H-dibenzo[2,3,6,7]-oxepino[4,5-c]pyrrole). On the basis of the MS-MS analyses of this fraction resulted in the main ions $m/z$ 316/318 and 314/316 (positive and negative mode), which corresponded to the [M $+ \text{H}^+$] and [M $- \text{H}^-$], respectively, of the 11-hydroxy-$N$-formylasenapine. In addition, some unknown, unrelated aliphatic compounds were
present. The product ion spectrum (MS/MS) of $m/z$ 316 was in agreement with that of Org 213979-0 and showed a.o. the loss of 28 Da (CO) and 71 Da (C$_6$H$_5$NCO).

**F11:** 6-hydroxy-$N$-formylasenapine. LC-ES-MS analyses of this fraction resulted in the main ions $m/z$ 316/318 and 314/316 (positive and negative mode). The product ion spectrum (MS/MS) of $m/z$ 316 also showed the loss of 28 (CO) and 71 Da (C$_6$H$_5$NCO) but was not identical to that of Org 213979-0 (11-hydroxy-$N$-formylasenapine). The product ion spectrum also eluted at a different retention time and probably
has the same structure as Org 213979-0 (F10), with only the hydroxyl
group at a different position. The $^1$H NMR spectra of peak F11 were
compared with the NMR spectra of N-formylasenapine, 11-hydroxy-
$N$-formylasenapine, and 7-hydroxyasenapine. For the aromatic pro-
tons, no coupling appeared to be present between H4 and H6 at 6.71
and 6.73 ppm, as found for the 7-hydroxyasenapine reference. There-
fore, this metabolite is tentatively identified as trans-5-chloro-2-
formyl-6-hydroxy-2,3,3$a$,12b-tetrahydro-1H-dibenzo[2,3,6,7]-ox-
epino[4,5-c]pyrrole.

**Discussion**

The objective of this study was to investigate the excretion mass
balance and metabolic profiling after sublingual administration of a
single 10-mg dose of $^{14}$C-labeled asenapine under steady-state con-
ditions in healthy male volunteers and to compare results with those
from the preclinical species used in safety assessment.

Mass balance resulted in a mean overall recovery of 93% of the
administered radioactivity, of which >80% was excreted within 96 h.
Excretion in the urine and feces accounted for 53 and 39%, respectiv-
ely.

Plasma concentrations of total radioactivity greatly exceeded those
of asenapine at all time points, indicating that asenapine is metabo-
lized extensively and rapidly. Our results showed that the asenapine
$N^\ominus$-glucuronide accounted for most of the radioactivity in plasma,
followed by the $N$-desmethyl carbamoyl glucuronide. The three other
radioactive detectable circulating compounds were asenapine, $N$-des-
methylasenapine, and asenapine 11-hydroxysulfate. All other metabolites, as identified in urine and feces, were either not present or below the limit of radioactivity detection and/or identification. Asenapine N-oxide was detected bioanalytically in some plasma samples (0.5 h up to 1.5 h postdose) at very low levels (maximally, 0.2 ng/ml) but was below the limit of radioactivity detection in all plasma samples analyzed.

Preclinical data on file showed that the basic amine functionality is essential for the pharmacological activity of asenapine. Asenapine N'-glucuronide and N-desmethylasenapine have diminished potency toward the receptors to which asenapine binds. Although N-desmethyl-N-carbamoyl-asenapine glucuronide was not tested for activity, the fact that the nitrogen is no longer basic would lead to loss of binding activity. 11-Hydroxyasenapine sulfate was also tested and showed a receptor-binding profile similar to that of asenapine. However, it was shown in the rat that this metabolite does not penetrate the brain. Other putative metabolites that were tested for receptor activity included asenapine N-oxide, 11-hydroxyasenapine, and 7-hydroxyasenapine (data on file, 2008). These metabolites were below the limit of radioactivity detection in human circulation, and, thus, it is unlikely that these metabolites contribute toward the pharmacological effects of asenapine. These data suggest that circulating human metabolites of asenapine do not contribute to the pharmacological effects of asenapine.

Metabolites observed were mainly derived from reactions at the nitrogen in the five-membered ring of asenapine. The primary route is direct glucuronidation. The main enzyme responsible for the direct glucuronidation of asenapine is UDP glucuronosyltransferase 1A4 (data on file, 2008). The biotransformation of quaternary ammonium N'-glucuronidation has been characterized in humans for more than 30 drugs and xenobiotics, including antihistamines, antidepressants, and antipsychotics (Hawes, 1998).

Glucuronidation is generally considered to be a pathway of detoxification, which commonly transforms lipophilic compounds into hydrophilic metabolites. Quaternary ammonium N'-glucuronides are not associated with toxicity. Asenapine N'-glucuronide is not suspected to cause any adverse effects because 1) it would not be expected to be chemically reactive and is a stable compound, 2) it is not pharmacologically active and would not contribute to suprapharmacological effects, 3) it is present to some extent in animal species used in safety assessments after administration of asenapine, and 4) asenapine is doseably maximally at 20 mg/day, resulting in low levels of circulating N'-glucuronide (<1 μM). Moreover, asenapine N'-glucuronide is of a class of metabolites that have been shown to be present in humans for other drugs at much greater levels than for asenapine (Hawes, 1998).

Also important but apparently less pronounced is the demethylation pathway, followed by association with carbamic acid and conjugation with glucuronic acid, resulting in the N-desmethyl carbamoyl glucuronide. It is not unreasonable to propose that the carbamic acid of asenapine serves as the transiently formed intermediate for glucuronidation. For several drugs containing a primary, secondary, or tertiary amine, the formation of a carbamic acid followed by glucuronidation has been observed (Ronfeld et al., 1982; Straub et al., 1988; Brown et al., 1990; Delbressine et al., 1990; Obach et al., 2005, 2006; Schaefer, 2006). An unprotonated primary or secondary amine and CO₂ are required to form a carbamic acid (Delbressine et al., 1990), which was subsequently conjugated with the glucuronic acid (Schaefer, 2006). The formation of a carbamoyl glucuronide has been observed in excreta or in the circulatory system of several species, including humans, for several drugs, including tocainide (Ronfeld et al., 1982), sertraline (Obach et al., 2005), rimantadine (Brown et al., 1990), and varenicline (Obach et al., 2006). Some drugs that contained tertiary amines, for instance, 6-chloro-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine (SK&F 86466) (Straub et al., 1988) and mirtazapine (Delbressine et al., 1990), are initially N-dealkylated to yield a secondary amine that is subsequently metabolized to a carbamate glucuronide.

The N'-glucuronide and the carbamoylglucuronide formation are probably due to the nucleophilic nature of the nitrogen in asenapine. It is instructive to compare these human biotransformation pathways of asenapine with those of mirtazapine and mianserin, drugs with a structure similar to that of asenapine. All three form the N'-glucuronide, whereas the carbamoylglucuronide was found only for mirtazapine and asenapine. These biotransformation routes are more dominantly present for asenapine, followed by mirtazapine and then mianserin, in line with the nucleophilic character of the nitrogen (asenapine > mirtazapine > mianserin). Moreover, because of this chemical property, formation of the N'-oxide metabolites as found in some urine and fecal samples is either generated by an enzymatically catalyzed process or formed spontaneously during the sample treatment.

Asenapine metabolism includes oxidative (phase 1) reactions at various positions next to reactions at the nitrogen. Formation of phase I metabolites, as investigated with individual cDNA-expressed major human cytochrome P450s (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) incubated with asenapine and human liver microsomes incubated with asenapine and specific enzyme inhibitors (data on file, 1998), was shown to be catalyzed mainly by CYP1A2 with contributions of CYP3A4 and CYP2D6. In humans, the 11-hydroxylated asenapine metabolite was mainly present in the conjugated form. The monohydroxylated intermediate could be further hydroxylated into a 10,11-dihydroxy intermediate. This diol was conjugated at either the 10- or 11-position, occasionally combined with methylation of the remaining hydroxy moiety. The formation of all these regio-isomeric conjugates, the glucuronides present as dia-stereoisomers, in combination with demethylation or N-oxidation resulted in many structurally related metabolites, which, under the conditions applied, could hardly be separated. Spectral (MS and NMR) analyses often showed that the HPLC fractions consisted of a mixture of metabolites. For that reason and because of the lack of corresponding reference compounds, no attempts were made to quantify the individual metabolites formed from asenapine.

Metabolites observed in humans were also observed in laboratory animals at least on the basis of retention time comparison (data on file; von dem Wildenberg et al., 1990). In addition, all metabolic pathways as observed in humans have been observed in preclinical species, which confirms that the choice of preclinical species used in safety assessment was acceptable.

In summary, the overall disposition and metabolism of asenapine has been determined in male volunteers. Asenapine, after a single sublingual radioiodolated dose under steady-state conditions, is absorbed rapidly and substantially. Total excretion averaged 90% with excretion being slightly higher in urine than in feces. Metabolism appeared to be extensive and resulted in several regio-isomeric hydroxylated and conjugated metabolites. The metabolic reactions occurred mainly at the nitrogen, resulting in direct glucuronidated and demethylated metabolites, which do not contribute to the pharmacological effects of asenapine.

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Authorship Contributions

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**Contributed new reagents or analytic tools:** Kemperman.

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References


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