Supplementary material

An Ex Vivo Model for Evaluating Blood–Brain Barrier Permeability, Efflux, and Drug Metabolism.

Karin Hellman[a], Peter Aadal Nielsen[b], Fredrik Ek[a], Roger Olsson[a*]

[a] Chemical Biology & Therapeutics unit, Department of Experimental Medical Science, Lund University, Lund, Sweden

[b] Department of Pharmacy, University of Copenhagen, Denmark

1) Test of brain barrier integrity using atenolol
2) Metabolic ID report performed by Admescope

Test of brain barrier integrity using atenolol

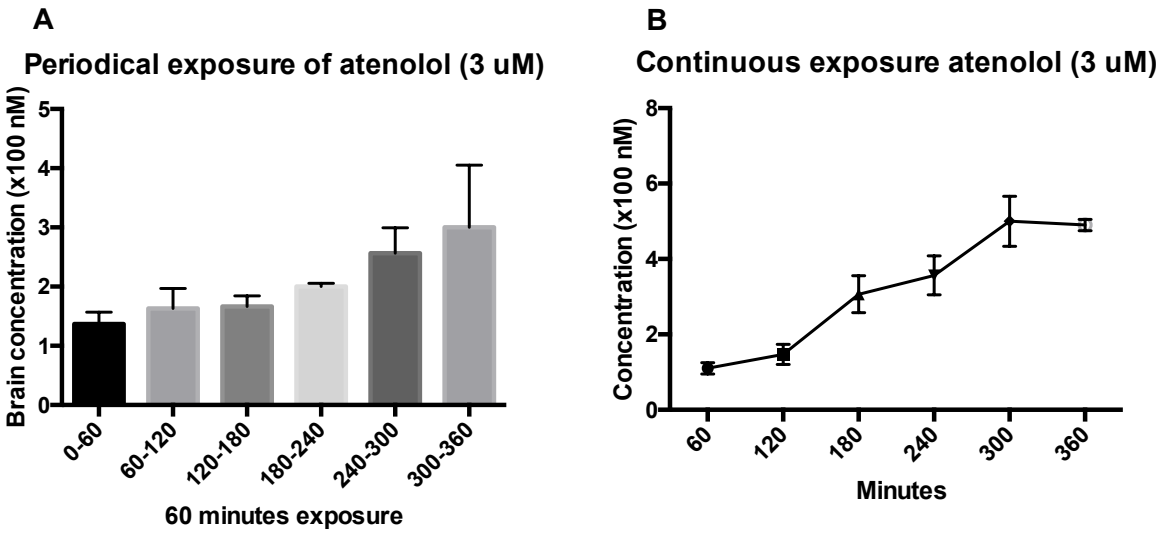

Figure 1S. Brain barrier integrity experiments using atenolol 3 μM. Using periodical exposure time 60 minutes intervals (A) and continuous exposure 0-360 minutes (B). Values represent the mean ± SD of three independent experiments (n = 3), two locust brains pooled in each experiment, from one male and one female. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. No statistically significant differences from 0-60 minutes was found.

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Final report 19 October 2015

METABOLITE IDENTIFICATION FOR CLOZAPINE AND MIDAZOLAM IN GRASSHOPPER BRAIN

Admescope Study Number ADM-15-1042

Ari Tolonen, PhD
Heidi Hautajärvi, MSc

Testing Facility:
Admescope Ltd, Oulu, Finland

Sponsor:
Lund University, Sweden

CONFIDENTIAL

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AUTHENTICATION

I confirm that this study is performed under my supervision and the results presented here are reported accurately according to the obtained data and observations.

Ari Tolonen, PhD
Study Director, Admescope Ltd

Date 19/10/2015

SUMMARY

Metabolite profiles of clozapine and midazolam n Grasshopper brain incubations (performed by the Sponsor) were analyzed using UPLC/QE-orbitrap-MS optimized for the purpose.

For clozapine, totally 18 metabolites (M1 – M18) were observed. Four oxidated/hydroxylated metabolites were observed, two with reaction sites in chlorobenzodiazepine (M1, M2) and two in methylpiperazine (M3, M4). Also, a further hydrogenation of M1/M2 in chlorobenzodiazepine (M5) was observed, as well as a combination of M1/M2 and M3/M4 with a further dehydrogenation (M6). Yet, phase I metabolites formed via N-demethylation (M7), a loss of C2H2 (M8), combination of M7 and M8, (M9) were observed. The conjugative metabolism consisted of piperazine methylation (M10), further acetylation of M7 (M14), glucoside conjugations directly to clozapine (M12, M13) or further to M7 (M15, M16), nitrile formation (M17, M18) to piperazine, and direct S-glutathione conjugation (M11). The reaction mechanism of M11 probably includes epoxide intermediate in benzodiazepine-ring-system.

N-demethylation (M7) was clearly the most abundant clozapine metabolite, and direct glucoside conjugation (M13) the second most abundant metabolite for clozapine in grasshopper brain. Metabolites M2, M3, M5, M7, M8, M10, M12 and M13 were observed in both brain and incubation well samples, while the metabolites M1, M4, M6, M9, and M14 - M18 were not observed in any of the incubation well samples. The relative abundance of metabolites with respect to clozapine was decreasing as a function of test concentration, suggesting saturation of the metabolic enzymes.

For midazolam, 14 metabolites were observed. Well known hydroxymetabolites 4-hydroxymidazolam (M1) and 1-hydroxymidazolam (M2)) were observed, together with metabolites hydroxylated/oxidated in methylimidazole (M3, M4). Methylimidazole was the site of most of the other metabolic reactions observed, including further hydrogenation of M1 (M7), further hydrogenation of M2/M3/M4 (M8), further oxidation and hydrogenation of M2/M3/M4 (M9, M10, M11), and hydroxylation in chlorodihydrobenzodiazepine with further hydrogenation in methylimidazole (M6). In addition, dioxidated metabolite M5 (site not known) was observed, as well as reductive defluorination (M12), and glucoside conjugations directly to midazolam (M13) and after hydroxylation and hydrogenation (M7, M8 or M9) (M14).

The metabolite profile for midazolam did not show very clear main metabolites. However, the glucoside conjugation M13, together with hydroxylated/oxidated M1 – M3, oxidated and hydrogenated M6 and M7, and dioxidated and hydrogenated M9 - M11 were the most abundant metabolites observed in grasshopper brain, their relative abundances changing a bit as a function of incubation concentrations. Almost all of the observed metabolites were present in both brain and incubation well samples in all test concentrations. Only M5 and M14 were not observed in any of the incubation well samples. Similarly to clozapine, the relative abundance of metabolites with respect to midazolam was decreasing as a function of incubation concentration, suggesting saturation of enzymatic reactions.

1. IMPLEMENTATION OF THE STUDY

1.1 Test site address

Admescope Ltd, Typpitie 1, FI-90620 Oulu, Finland.

1.2 Admescope personnel

Study Director Ari Tolonen, Investigator Heidi Hautajärvi, Laboratory Technician Marika Loukkola.

1.3 Dates of the study

The work was conducted between October 8th - 9th 2015.

1.4 Qualification

The study is a non-GLP study. Quality system and SOPs of Admescope Ltd are used in all procedures.

2. MATERIALS AND METHODS

2.1 Chemicals and suppliers

HPLC grade methanol and acetonitrile: Merck (Darmstadt, Germany)

HPLC grade formic acid, acetic acid and ammonium formate: BDH Laboratory Supplies (Poole, UK)

Other chemicals: Sigma Aldrich (Helsinki, Finland), the highest purity available.

Water was in-house freshly prepared with a Direct-Q3 (Millipore Oy, Espoo, Finland) purification system and UP grade (ultra pure, 18.2 MΩ).

2.2 Sample preparation for analysis

Incubations were performed by the Sponsor. Briefly, 1, 3 and 10 µM test concentrations were used for both test compounds, after which brains (from two grasshoppers) were combined and mixed with 40 µl of acetonitrile, which was then shipped for Admescope in dry ice. In addition, samples from the incubation well were collected (after removal of brains) and shipped together with the actual brain samples. At Admescope, the brain samples were thawed at room temperature (RT) and20 µl of UP-grade water was added, after which the samples were ultrasonicated for 20 min, centrifuged (10 min at 13000 × g (Heraeus Pico 17 centrifuge), and the supernatants were collected for the analysis. The test solution samples were thawed at room temperature (RT), centrifuged (10 min at 13000 × g (Heraeus Pico 17 centrifuge), and the supernatants were pipetted to Vials to wait for analysis.

2.3 Liquid chromatography-mass spectrometry

Instrumentation	Thermo U3000 UHPLC + Q-Exactive Orbitrap mass spectrometer + PDA detector
Column	Waters Acquity BEH C18 (2.1 × 50 mm, 1.7 µm) column with guard filter
Polarity	ESI +
Sheath Gas	nitrogen 55 units
Auxiliary Gas	nitrogen 3 units
Sweep gas	nitrogen 2 units
Spray voltage	1500 V
Capillary temperature	350 (°C)
Aux gas heater temp	500 (°C)
Mass range	m/z 100 – 1200
Acquisition time	7 Hz for full scan, IT 100 ms for DDI MS/MS
Resolution	35 000 (FWHM @ m/z 200) for full scan, 17 500 for MS/MS in DDI mode
Collision energy	off for full scan; 45 units for DDI MS/MS (inclusion list for expected metabolites ON; also other unexpected most abundant metabolites chosen for MS/MS)
Calibration	External
Software	Thermo Xcalibur 3.0.63
Ion chromatographic window	5 ppm

Gradient Elution; A = 0.1% formic acid, B = acetonitrile

Time	Flow	A%	B%
0.00	0.500 ml/min	98	2
0.50	0.500 ml/min	98	2
3.00	0.500 ml/min	40	60
4.00	0.500 ml/min	10	90
5.00	0.500 ml/min	98	2

Temperature	35 (°C)
Injection volume	4 µl

Ion chromatograms were extracted from the total ion chromatograms using calculated monoisotopic accurate masses with 5 mDa window. The metabolites were mined from the data using software-aided data processing (Thermo Compound Discoverer including structure-intelligent dealkylation tool & mass defect filter) with manual confirmation

3. RESULTS

The LC/MS data obtained from clozapine, midazolam, and their metabolites are summarised in Tables 1 and 2, and ion chromatograms are shown in the Appendices I and II. All the detected metabolites were tentatively identified according to the accurate mass data, retention times, and high resolution fragment ion data. The tentative identifications for the metabolite structures are shown in the Figures 1 - 2; whereas the fragment ion assignment is shown in Appences III – IV. The metabolite profiles in all analysed samples, expressed as a percentage of each metabolite from total LC/MS peak area, are shown in Appences V – VI. It is worth stressing that identical LC/MS response for metabolites and parent compounds is assumed, which probably is not the case.

For clozapine, totally 18 metabolites (M1 – M18) were observed. Four oxidated/hydroxylated metabolites were observed, two with reaction sites in chlorobenzodiazepine (M1, M2) and two in methylpiperazine (M3, M4). Also, a further hydrogenation of M1/M2 in chlorobenzodiazepine (M5) was observed, as well as a combination of M1/M2 and M3/M4 with a further dehydrogenation (ketone formation) in methylpiperazine (M6). The fragment ion data for M6 is however not unambiguous, and alternatively it may be that the dehydrogenation is not in methylpiperazine but rather as a quinone-imine formation to benzodiazepine (after hydroxylation to carbon adjacent to chlorine-binding carbon). Yet, phase I metabolites formed via N-demethylation (M7), a loss of C2H2 (M8), combination of M7 and M8, (M9) were observed. The conjugative metabolism consisted of piperazine methylation (M10), further acetylation of M7 (M14), glucoside conjugations directly to clozapine (M12, M13) or further to M7 (M15, M16),nitrile formation (M17, M18) to piperazine, and direct S-glutathione conjugation (M11). The reaction mechanism of M11 probably includes epoxide intermediate in benzodiazepine-ring-system, and it is worth noticing that also three additional LC/MS peaks with exact mass fitting to S-glutatione conjugates were observed, but their abundance was very low and no supporting MS/MS data was obtained (see M11 ion chromatogram in Appendix I). In addition, similar very minor LC/MS peak with intensity close to detection limit was observed for a m/z fitting to exact mass of GSH-conjugation replacing chlorine atom (m/z 598.2442), as well as replacement of chlorine by S-thiomethyl (m/z 339.1638) but no confirming MS/MS data was obtained. Formation mechanism of M17 – M18 is bit unclear; typically this reaction is known to occur for iminium-type reactive metabolites after trapping/stabilizing with cyanide.

N-demethylation (M7) was clearly the most abundant clozapine metabolite, and direct glucoside conjugation (M13) the second most abundant metabolite for clozapine in grasshopper brain. Both M7 and M13 were also observed in the samples collected from wells after the incubation, although the relative amounts were significantly lower compared to the actual brain samples. Also metabolites M2, M3, M5, M8, M10 and M12 were observed in both brain and incubation well samples, while the metabolites M1, M4, M6, M9, and M14 - M18 were not observed in any of the incubation well samples. The relative abundance of metabolites with respect to clozapine was decreasing as a function of test concentration, suggesting saturation of the metabolic enzymes.

For midazolam, 14 metabolites were observed. Well known hydroxymetabolites 4-hydroxymidazolam (M1) and 1-hydroxymidazolam (M2)) were observed, together with metabolites hydroxylated/oxidated in methylimidazole (M3, M4). Identification of M2 was confirmed by using in-house standard for 1-hydroxymidazolam. Methylimidazole was the site of most of the other metabolic reactions observed, including further hydrogenation of M1 (M7), further hydrogenation of M2/M3/M4 (M8), further oxidation and hydrogenation of M2/M3/M4 (M9, M10, M11), and hydroxylation in chlorodihydrobenzodiazepine with further hydrogenation in methylimidazole (M6). In addition, dioxidated metabolite M5 (site not known) was observed, as well as reductive defluorination (M12), and glucoside conjugations directly to midazolam (M13) and after hydroxylation and hydrogenation (M7, M8 or M9) (M14).

The metabolite profile for midazolam did not show very clear main metabolites. However, the glucoside conjugation M13, together with hydroxylated/oxidated M1 – M3, oxidated and hydrogenated M6 and M7, and dioxidated and hydrogenated M9 - M11 were the most abundant metabolites observed in grasshopper brain, their relative abundances changing a bit as a function of incubation concentrations. Almost all of the observed metabolites were present in both brain and

incubation well samples in all test concentrations, although the amounts were significantly lower in well samples compared to the actual brain samples. Only M5 and M14 were not observed in any of the incubation well samples and M8 was observed in all other samples but not in 1 µM incubation well samples. Similarly to clozapine, the relative abundance of metabolites with respect to midazolam was decreasing as a function of incubation concentration, suggesting saturation of enzymatic reactions.

Table 1. LC/ESI/TOF-MS data obtained for Clozapine and its metabolites.

Comp	Identification	RT min	ion	meas (m/z)	calc (m/z)
	Clozapine	**3.13**	**C18H19ClN4**	**327.1365**	**327.1371**
	Fragment (loss of CH5N)		[C17H15ClN3]+	296.0945	296.0949
	Fragment (loss of C3H7N)		[C15H13ClN3]+	270.0788	270.0793
	Fragment (loss of C4H10N)		[C14H10ClN3]+	255.0548	255.0558
	Fragment (loss of C3H7N + NH3)		[C15H10ClN2]+	253.0529	253.0527
	Fragment (loss of C4H5N + NH3)		[C14H12ClN2]+	243.0683	243.0684
	Fragment (loss of C3H7N + Cl)		[C15H13N3]+	235.1099	235.1104
	Fragment (loss of C5H12N2)		[C13H8ClN2]+	227.0367	227.0371
	Fragment (loss of C4H10N + N + Cl)		[C14H10N2]+	206.0840	206.0838
	Fragment (loss of C5H12N2 + Cl)		[C13H8N2]+	192.0678	192.0682
	Fragment (loss of CH2 + C13H9ClN2)		[C4H9N2]+	85.0766	85.0760
	Fragment (loss of C13H10ClN3)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C13H12ClN3)		[C5H8N]+	82.0657	82.0651
	Fragment (loss of C15H12ClN3)		[C3H8N]+	58.0660	58.0651
M1	**Oxidation in chlorodibenzodiazepine**	**2.26**	**C18H19ClN4O**	**343.1317**	**343.1320**
	Fragment (loss of C3H7N)		[C15H13ClN3O]+	286.0738	286.0742
	Fragment (loss of C5H12N2)		[C13H8ClN2O]+	243.0310	243.0320
	Fragment (loss of C13H10ClN3O)		[C5H10N]+	84.0812	84.0808
	Fragment (loss of C14H10ClN3O)		[C4H10N]+	72.0815	72.0808
	Fragment (loss of C14H12ClN3O)		[C4H8N]+	70.0658	70.0651
M2	**Oxidation in chlorodibenzodiazepine**	**2.85**	**C18H19ClN4O**	**343.1316**	**343.1320**
	Fragment (loss of CH5N)		[C17H15ClN3O]+	312.0893	312.0898
	Fragment (loss of C3H7N)		[C15H13ClN3O]+	286.0739	286.0742
	Fragment (loss of C5H12N2)		[C13H8ClN2O]+	243.0308	243.0320
	Fragment (loss of C5H12N2 + Cl)		[C13H8N2O]+	208.0627	208.0631
	Fragment (loss of C13H10ClN3O)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C14H12ClN3O)		[C4H8N]+	70.0659	70.0651
M3	**Oxidation in methylpiperazine**	**3.31**	**C18H19ClN4O**	**343.1318**	**343.1320**
	Fragment (loss of C2H4O)		[C16H16ClN4]+	299.1053	299.1058
	Fragment (loss of CH5NO)		[C17H15ClN3]+	296.0945	296.0949
	Fragment (loss of C2H4O + NH3)		[C16H13ClN3]+	282.0788	282.0793
	Fragment (loss of C3H7NO)		[C15H13ClN3]+	270.0802	270.0793
	Fragment (loss of C3H9NO)		[C15H11ClN3]+	268.0635	268.0636
	Fragment (loss of C5H10NO)		[C13H10ClN3]+	243.0553	243.0558
	Fragment (loss of C5H12N2O)		[C13H8ClN2]+	227.0368	227.0371
	Fragment (loss of C5H12N2O + Cl)		[C13H8N2]+	192.0680	192.0682
	Fragment (loss of C13H10ClN3)		[C5H10NO]+	100.0762	100.0757
	Fragment (loss of C13H7ClN2 + H2O)		[C5H11N2]+	99.0922	99.0917
	Fragment (loss of CH2 + C13H9ClN2O)		[C4H9N2]+	85.0766	85.0760
	Fragment (loss of C13H12ClN3O)		[C5H8N]+	82.0657	82.0651
	Fragment (loss of C15H12ClN3)		[C3H8NO]+	74.0607	74.0600
	Fragment (loss of C14H12ClN3O)		[C4H8N]+	70.0657	70.0651
	Fragment (loss of C15H12ClN3O)		[C3H8N]+	58.0659	58.0651
	Fragment (loss of C15H14ClN3O)		[C3H6N]+	56.0503	56.0495
M4	**Oxidation in methylpiperazine**	**3.46**	**C18H19ClN4O**	**343.1318**	**343.1320**
	Fragment (loss of CO)		[C17H20ClN4]+	315.1371	315.1371
	Fragment (loss of C4H7NO)		[C14H13ClN3]+	258.0791	258.0793
	Fragment (loss of C5H12N2O)		[C13H8ClN2]+	227.0352	227.0371
	Fragment (loss of C4H7NO + HCl)		[C14H12N3]+	222.1024	222.1026
	Fragment (loss of C5H12N2O2 + Cl)		[C13H8N2]+	192.0685	192.0682
	Fragment (loss of C13H10ClN3O)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C15H12ClN3O)		[C3H8N]+	58.0659	58.0651
M5	**Oxidation + hydrogenation in chlorodibenzodiazepine**	**2.43**	**C18H21ClN4O**	**345.1473**	**345.1477**
	Fragment (loss of C5H12N2)		[C13H10ClN2O]+	245.0473	245.0476
	Fragment (loss of C5H12N2 + Cl)		[C13H10N2O]+	210.0779	210.0788
	Fragment (loss of C5H12N2 + HCl)		[C13H9N2O]+	209.0706	209.0709

	Fragment (loss of C13H9ClN2O)		[C5H13N2]+	101.1077	101.1073
	Fragment (loss of C13H7ClN2 + H2O)		[C5H11N2]+	99.0921	99.0917
	Fragment (loss of C13H10ClN3O)		[C5H10N]+	84.0814	84.0808
	Fragment (loss of C15H12ClN3)		[C3H8NO]+	74.0607	74.0600
	Fragment (loss of C14H12ClN3O)		[C4H8N]+	70.0658	70.0651
	Fragment (loss of C15H12ClN3O)		[C3H8N]+	58.0659	58.0651
M6	**Oxidation + dehydrogenation in methylpiperazine + oxidation in chlorodibenzodiazepine**	**3.38**	**C18H17ClN4O2**	**357.1109**	**357.1113**
	Fragment (loss of C2H3NO)		[C16H15ClN3O]+	300.0883	300.0898
	Fragment (loss of C3H6NO)		[C15H12ClN3O]+	285.0666	285.0663
	Fragment (loss of C12H7ClN2O)		[C6H11N2O]+	127.0867	127.0866
	Fragment (loss of C13H7ClN2O2)		[C5H11N2]+	99.0921	99.0917
	Fragment (loss of C13H8ClN2O2)		[C5H10N2]+	98.0842	98.0838
	Fragment (loss of C14H9ClN2O2)		[C4H9N2]+	85.0765	85.0760
	Fragment (loss of C14H10ClN2O2)		[C4H8N2]+	84.0687	84.0682
	Fragment (loss of C13H10ClN3O2)		[C5H8N]+	82.0656	82.0651
	Fragment (loss of C14H10ClN3O2)		[C4H8N]+	70.0659	70.0651
M7	**N-Demethylation**	**2.95**	**C17H17ClN4**	**313.1209**	**313.1215**
	Fragment (loss of H2)		[C17H16ClN4]+	311.1054	311.1058
	Fragment (loss of NH3)		[C17H15ClN3]+	296.0946	296.0949
	Fragment (loss of C2H5N)		[C15H13ClN3]+	270.0789	270.0793
	Fragment (loss of C2H5N + NH3)		[C15H10ClN2]+	253.0523	253.0527
	Fragment (loss of C4H7N)		[C13H11ClN3]+	244.0633	244.0636
	Fragment (loss of C2H5N + Cl)		[C15H13N3]+	235.1098	235.1104
	Fragment (loss of C4H10N2)		[C13H8ClN2]+	227.0367	227.0371
	Fragment (loss of C4H11N2)		[C13H7ClN2]+	226.0289	226.0292
	Fragment (loss of C4H10N2 + Cl)		[C13H8N2]+	192.0680	192.0682
	Fragment (loss of C13H9ClN2)		[C4H9N2]+	85.0766	85.0760
	Fragment (loss of C14H11ClN2)		[C3H7N2]+	71.0609	71.0604
	Fragment (loss of C13H10ClN3)		[C4H8N]+	70.0658	70.0651
M8	**Loss of C2H2 in methylpiperazine**	**2.46**	**C16H17ClN4**	**301.1213**	**301.1215**
	Fragment (loss of CH5N)		[C15H13ClN3]+	270.0790	270.0793
	Fragment (loss of CH5N + C2H2)		[C13H11ClN3]+	244.0629	244.0636
	Fragment (loss of C3H10N2)		[C13H8ClN2]+	227.0369	227.0371
	Fragment (loss of C3H10N2 + Cl)		[C13H8N2]+	192.0678	192.0682
	Fragment (loss of C6H7ClN2)		[C10H11N2]+	159.0916	159.0917
	Fragment (loss of C11H6ClN3)		[C5H12N]+	86.0968	86.0964
	Fragment (loss of C12H10ClN3)		[C4H8N]+	70.0659	70.0651
	Fragment (loss of C13H10ClN3)		[C3H8N]+	58.0659	58.0651
M9	**Demethylation + Loss of C2H2 in methylpiperazine**	**2.40**	**C15H15ClN4**	**287.1057**	**287.1058**
M10	**Methylation in methylpiperazine**	**3.19**	**C19H21ClN4**	**341.1517**	**341.1528**
	Fragment (loss of C2H4)		[C17H18ClN4]+	313.1206	313.1215
	Fragment (loss of C2H7N)		[C17H15ClN3]+	296.0941	296.0949
	Fragment (loss of C4H9N)		[C15H13ClN3]+	270.0790	270.0793
	Fragment (loss of C6H14N2)		[C13H8ClN2]+	227.0368	227.0371
	Fragment (loss of C6H14N2 + Cl)		[C13H8N2]+	192.0682	192.0682
	Fragment (loss of C15H11ClN2)		[C4H11N2]+	87.0922	87.0917
	Fragment (loss of C15H14ClN3)		[C4H8N]+	70.0658	70.0651
M11	**S-Glutatione conjugation**	**2.73**	**C28H34ClN7O6S**	**632.2051**	**632.2053**
	Fragment (loss of C10H16N3O6)		[C18H19ClN4S]+	359.1072	359.1092
	Fragment (loss of C3H7N + C10H15N3O6)		[C15H13ClN3S]+	302.0510	302.0513
	Fragment (loss of C10H15N3O6S + C13H10ClN3)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C10H15N3O6S + C14H10ClN3)		[C4H10N]+	72.0815	72.0808
	Fragment (loss of C10H15N3O6S + C14H12ClN3)		[C4H8N]+	70.0658	70.0651
	Fragment (loss of C10H15N3O6S + C15H14ClN3)		[C3H6N]+	56.0503	56.0495
M12	**Glucoside conjugation**	**2.80**	**C24H29ClN4O5**	**489.1898**	**489.1899**
	Fragment (loss of C3H7N + H4O2)		[C21H19ClN3O3]	396.1111	396.1109
	Fragment (loss of C6H10O5)		[C18H20ClN4]+	327.1382	327.1371
	Fragment (loss of C6H10O4 + CH5N)		[C17H15ClN3O]+	312.0893	312.0893

	Fragment (loss of C6H10O5 + CH5N)		[C17H15ClN3]+	296.0950	296.0949
	Fragment (loss of C6H10O5 + C2H4 + NH3)		[C16H13ClN3]+	282.0811	282.0793
	Fragment (loss of C6H10O5 + C3H7N)		[C15H13ClN3]+	270.0788	270.0793
	Fragment (loss of C6H10O5 + C3H9N)		[C15H11ClN3]+	268.0635	268.0636
	Fragment (loss of C6H10O5 + C4H9N)		[C14H11ClN3]+	256.0623	256.0636
	Fragment (loss of C6H10O5 + C4H7N + NH3)		[C14H10ClN2]+	241.0527	241.0527
	Fragment (loss of C6H10O5 + C5H12N2)		[C13H8ClN2]+	227.0363	227.0371
	Fragment (loss of C6H10O5 + C13H7ClN2)		[C5H13N2]+	101.1078	101.1073
	Fragment (loss of C6H10O5 + C13H10ClN3)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C6H10O5 + C14H10ClN3)		[C4H10N]+	72.0815	72.0808
	Fragment (loss of C6H10O5 + C14H12ClN3)		[C4H8N]+	70.0658	70.0651
	Fragment (loss of C6H10O5 + C15H12ClN3)		[C3H8N]+	58.0659	58.0651
M13	**Glucoside conjugation**	**3.01**	**C24H29ClN4O5**	**489.1896**	**489.1899**
	Fragment (loss of C6H10O5)		[C18H20ClN4]+	327.1367	327.1371
	Fragment (loss of C6H10O5 + CH5N)		[C17H15ClN3]+	296.0946	296.0949
	Fragment (loss of C6H10O5 + C3H7N)		[C15H13ClN3]+	270.0789	270.0793
	Fragment (loss of C6H10O5 + C5H12N2)		[C13H8ClN2]+	227.0368	227.0371
	Fragment (loss of C6H10O5 + C5H12N2 + Cl)		[C13H8N2]+	192.0680	192.0682
	Fragment (loss of C6H10O5 + C13H10ClN3)		[C5H10N]+	84.0813	84.0808
	Fragment (loss of C6H10O5 + C15H12ClN3)		[C3H8N]+	58.0659	58.0651
M14	**N-Demethylation + Acetylation in methylpiperazine**	**3.07**	**C19H19ClN4O**	**355.1313**	**355.1320**
	Fragment (loss of CH2)		[C18H18ClN4O]+	341.1158	341.1164
	Fragment (loss of C2H2)		[C17H18ClN4O]+	329.1160	329.1164
	Fragment (loss of C2H2O)		[C17H18ClN4]+	313.1209	313.1215
	Fragment (loss of NH3 + C2H2O)		[C17H15ClN3]+	296.0957	296.0949
	Fragment (loss of C2H5N + C2H2O)		[C15H13ClN3]+	270.0787	270.0793
	Fragment (loss of C2H5N + NH3 + C2H2O)		[C15H10ClN2]+	253.0522	253.0527
	Fragment (loss of C4H7N + C2H2O)		[C13H11ClN3]+	244.0629	244.0636
	Fragment (loss of C4H10N2 + C2H2O)		[C13H8ClN2]+	227.0367	227.0371
	Fragment (loss of C4H10N2 + Cl + C2H2O)		[C13H8N2]+	192.0679	192.0682
	Fragment (loss of C13H10ClN3 + C2H2)		[C4H8NO]+	86.06052	86.0600
	Fragment (loss of C13H10ClN3 + C2H2O)		[C4H8N]+	70.06579	70.0651
M15	**Demethylation + Glucoside conjugation**	**2.68**	**C23H27ClN4O5**	**475.1741**	**475.1743**
M16	**Demethylation + Glucoside conjugation**	**2.84**	**C23H27ClN4O5**	**475.1744**	**475.1743**
	Fragment (loss of C6H10O5)		[C17H18ClN4]+	313.1217	313.1215
	Fragment (loss of C6H10O5 + NH3)		[C17H15ClN3]+	296.0943	296.0949
	Fragment (loss of C6H10O5 + C2H5N)		[C15H13ClN3]+	270.0789	270.0793
	Fragment (loss of C6H10O5 + C2H7N)		[C15H11ClN3]+	268.0639	268.0636
	Fragment (loss of C6H10O5 + C2H5N + NH3)		[C15H10ClN2]+	253.0522	253.0527
	Fragment (loss of C6H10O5 + C4H7N)		[C13H11ClN3]+	244.0634	244.0636
	Fragment (loss of C6H10O5 + C4H10N2)		[C13H8ClN2]+	227.0363	227.0371
	Fragment (loss of C6H10O5 + C4H10N2 + Cl)		[C13H8N2]+	192.0676	192.0682
	Fragment (loss of C6H10O5 + C13H10ClN3)		[C4H8N]+	70.06578	70.0651
M17	**Nitrile formation in methylpiperazine**	**3.35**	**C19H18ClN5**	**352.1320**	**352.1323**
	Fragment (loss of C4H6N2)		[C15H13ClN3]+	270.0793	270.0793
	Fragment (loss of C4H6N2 + C2H2)		[C13H11ClN3]+	244.0633	244.0636
	Fragment (loss of C6H11N3)		[C13H8ClN2]+	227.0367	227.0371
	Fragment (loss of C6H11N3 + Cl)		[C13H8N2]+	192.0681	192.0682
	Fragment (loss of C14H11ClN4)		[C5H8N]+	82.0657	82.0651
M18	**Nitrile formation in methylpiperazine**	**3.86**	**C19H18ClN5**	**352.1320**	**352.1326**
	Fragment (loss of CHN)		[C18H18ClN4]+	325.1211	325.1215
	Fragment (loss of C4H8N2)		[C15H11ClN3]+	268.0633	268.0636
	Fragment (loss of C4H6N2 + C2H2)		[C13H11ClN3]+	244.0632	244.0636
	Fragment (loss of C6H11N3)		[C13H8ClN2]+	227.0367	227.0371
	Fragment (loss of C6H11N3 + Cl)		[C13H8N2]+	192.0682	192.0682
	Fragment (loss of C14H11ClN4)		[C5H8N]+	82.0657	82.0651

Table 2. LC/ESI/TOF-MS data obtained for Midazolam and its metabolites.

Comp	Identification	RT min	ion	meas (m/z)	calc (m/z)
	Midazolam	3.40	C18H13ClFN3	326.0848	326.0855
	Fragment (loss of NH3)		[C18H11ClFN2]+	309.0584	309.0589
	Fragment (loss of HF)		[C18H13ClN3]+	306.0788	306.0793
	Fragment (loss of CHN)		[C17H13ClFN2]+	299.0735	299.0746
	Fragment (loss of Cl)		[C18H14FN3]+	291.1161	291.1166
	Fragment (loss of C2H3N)		[C16H11ClFN2]+	285.0585	285.0589
	Fragment (loss of HF + HCl)		[C18H12N3]+	270.1022	270.1026
	Fragment (loss of CHN + HCl)		[C17H12FN2]+	263.0979	263.0979
	Fragment (loss of C3H4N2)		[C15H10ClFN]+	258.0476	258.0480
	Fragment (loss of C2H3N + HCl)		[C16H10FN2]+	249.0818	249.0823
	Fragment (loss of C4H6N2)		[C14H8ClFN]+	244.0321	244.0324
	Fragment (loss of C6H5F)		[C12H9ClN3]+	230.0477	230.0480
	Fragment (loss of C3H4N2 + HCl)		[C15H9FN]+	222.0707	222.0714
	Fragment (loss of C7H4FN)		[C11H10ClN2]+	205.0525	205.0527
	Fragment (loss of C6H5F + C2H3N)		[C10H6ClN2]+	189.0214	189.0214
	Fragment (loss of C7H4FN + Cl)		[C11H10N2]+	170.0839	170.0838
	Fragment (loss of C13H7ClFN)		[C5H7N2]+	95.0609	95.0604
M1	**Hydroxylation in dihydrodiazepine (4-OH-MDZ)**	**3.20**	**C18H13ClFN3O**	**342.0799**	**342.0804**
	Fragment (loss of NH3)		[C18H11ClFN2O]+	325.0531	325.0538
	Fragment (loss of CHN)		[C17H13ClFN2O]+	315.0692	315.0695
	Fragment (loss of Cl)		[C18H14FN3O]+	307.1101	307.1115
	Fragment (loss of CHN + H2O)		[C17H11ClFN2]+	297.0585	297.0589
	Fragment (loss of C2H3NO)		[C16H11ClFN2]+	285.0589	285.0589
	Fragment (loss of C3H3NO)		[C15H11ClFN2]+	273.0586	273.0589
	Fragment (loss of C3H4N2O)		[C15H10ClFN]+	258.0482	258.0480
	Fragment (loss of C3H4N2O + Cl)		[C15H10FN]+	223.0789	223.0792
	Fragment (loss of C11H9ClN2O)		[C7H5FN]+	122.0402	122.0401
	Fragment (loss of C13H9ClFN)		[C5H5N2O]+	109.0397	109.0396
	Fragment (loss of C13H7ClFNO)		[C5H7N2]+	95.0608	95.0604
	Fragment (loss of C14H8ClFN2)		[C4H6NO]+	84.0449	84.0444
	Fragment (loss of C14H9ClFNO)		[C4H5N2]+	81.0454	81.0447
M2	**Hydroxylation in methylimidazole (1-OH-MDZ)**	**3.34**	**C18H13ClFN3O**	**342.0800**	**342.0804**
	Fragment (loss of H2O)		[C18H12ClFN3]+	324.0693	324.0698
	Fragment (loss of CHN)		[C17H13ClFN2O]+	315.0692	315.0695
	Fragment (loss of H2O + HF)		[C18H11ClN3]+	304.0632	304.0636
	Fragment (loss of CHN + H2O)		[C17H11ClFN2]+	297.0582	297.0589
	Fragment (loss of H2O + Cl)		[C18H12FN3]+	289.1003	289.1010
	Fragment (loss of CHN + H2O + Cl)		[C17H11FN2]+	262.0900	262.0901
	Fragment (loss of C7H4FN + H2O)		[C11H8ClN2]+	203.0369	203.0371
	Fragment (loss of C7H4FN + H2O +CHN)		[C10H7ClN]+	176.0261	176.0262
	Fragment (loss of C7H4FN + H2O + Cl)		[C11H8N2]+	168.0680	168.0682
	Fragment (loss of C11H8ClN3O)		[C7H6F]+	109.0450	109.0448
	Fragment (loss of C13H7ClFNO)		[C5H7N2]+	95.0607	95.0604
M3	**Oxidation in methylimidazole**	**3.51**	**C18H13ClFN3O**	**342.0799**	**342.0804**
	Fragment (loss of OH)		[C18H13ClFN3]+	325.0771	325.0777
	Fragment (loss of H2O)		[C18H12ClFN3]+	324.0691	324.0698
	Fragment (loss of CH2 + H2O)		[C17H10ClFN3]+	310.0536	310.0542
	Fragment (loss of C2H2O)		[C16H12ClFN3]+	300.0692	300.0698
	Fragment (loss of CHN + H2O)		[C17H11ClFN2]+	297.0585	297.0589
	Fragment (loss of HO + Cl)		[C18H13FN3]+	290.1087	290.1088
	Fragment (loss of C2H5NO)		[C16H9ClFN2]+	283.0429	283.0433
	Fragment (loss of C3H3NO)		[C15H11ClFN2]+	273.0586	273.0589
	Fragment (loss of C3H3NO + HF)		[C15H10ClN2]+	253.0525	253.0527
	Fragment (loss of C4H4N2O)		[C14H10ClFN]+	246.0478	246.0480
	Fragment (loss of C4H4N2O + Cl)		[C14H10FN]+	211.0789	211.0792
	Fragment (loss of C9H6FNO)		[C9H8ClN2]+	179.0369	179.0371

	Fragment (loss of C9H8FNO)		[C9H6ClN2]+	177.0211	177.0214
	Fragment (loss of C9H6ClNO)		[C9H8FN2]+	163.0664	163.0666
	Fragment (loss of C10H7FN2O)		[C8H7ClN]+	152.0260	152.0262
	Fragment (loss of C11H9ClN2O)		[C7H5FN]+	122.0402	122.0401
	Fragment (loss of C10H7FN2O + Cl)		[C8H7N]+	117.0574	117.0573
M4	**Oxidation in methylimidazole**	**3.74**	**C18H13ClFN3O**	**342.0800**	**342.0804**
	Fragment (loss of NH3)		[C18H11ClFN2O]+	325.0532	325.0538
	Fragment (loss of CH3N)		[C17H11ClFN2O]+	313.0532	313.0538
	Fragment (loss of C3H3NO)		[C15H11ClFN2]+	273.0585	273.0589
	Fragment (loss of C3H3NO + HF)		[C15H10ClN2]+	253.0524	253.0527
	Fragment (loss of C4H5N2O)		[C14H9ClFN]+	245.0397	245.0397
	Fragment (loss of C9H6FN)		[C9H8ClN2O]+	195.0318	195.0320
	Fragment (loss of C10H7FN2O + Cl)		[C8H7N]+	117.0574	117.0573
M5	**2x Oxidation**	**3.14**	**C18H13ClFN3O2**	**358.0744**	**358.0753**
M6	**Oxidation in chlorodihydrobenzodiazepine + hydrogenation in methylimidazole**	**2.56**	**C18H15ClFN3O**	**344.0958**	**344.0960**
	Fragment (loss of NH3)		[C18H13ClFN2O]+	327.0676	327.0695
	Fragment (loss of CH3N)		[C17H13ClFN2O]+	315.0689	315.0695
	Fragment (loss of CH3N + H2O)		[C17H11ClFN2]+	297.0585	297.0589
	Fragment (loss of C3H6N2)		[C15H10ClFNO]+	274.0430	274.0429
	Fragment (loss of C11H11FN2)		[C7H5ClNO]+	154.0052	154.0054
	Fragment (loss of C11H10ClN3O)		[C7H6F]+	109.0451	109.0448
	Fragment (loss of C13H9ClFNO)		[C5H7N2]+	95.0608	95.0604
	Fragment (loss of C14H8ClFN2O)		[C4H8N]+	70.0658	70.0651
M7	**Hydroxylation in benzodiazepine + Hydrogenation in methylimidazole**	**2.94**	**C18H15ClFN3O**	**344.0957**	**344.0960**
	Fragment (loss of NH3)		[C18H13ClFN2O]+	327.0681	327.0695
	Fragment (loss of H2O)		[C18H14ClFN3]+	326.0849	326.0855
	Fragment (loss of CH3N)		[C17H13ClFN2O]+	315.0695	315.0695
	Fragment (loss of NH3 + H2O)		[C18H11ClFN2]+	309.0584	309.0589
	Fragment (loss of CHN + H2O)		[C17H13ClFN2]+	299.0736	299.0746
	Fragment (loss of CH3N + H2O)		[C17H11ClFN2]+	297.0585	297.0589
	Fragment (loss of H2O + Cl)		[C18H14FN3]+	291.1168	291.1166
	Fragment (loss of H2O + NH3 + Cl)		[C18H11FN2]+	274.0891	274.0901
	Fragment (loss of CHN + H2O + Cl)		[C17H13FN2]+	264.1050	264.1057
	Fragment (loss of C5H6N)		[C13H10ClFNO]+	250.0419	250.0429
	Fragment (loss of C7H6FNO)		[C11H10ClN2]+	205.0524	205.0527
	Fragment (loss of C6H5F + C2H3N + H2O)		[C10H6ClN2]+	189.0210	189.0214
	Fragment (loss of C11H10ClN3O)		[C7H6F]+	109.0450	109.0448
	Fragment (loss of C13H9ClFNO)		[C5H7N2]+	95.0607	95.0604
M8	**Oxidation + Hydrogenation in methylimidazole**	**3.34**	**C18H15ClFN3O**	**344.0955**	**344.0960**
	Fragment (loss of CH3N)		[C17H13ClFN2O]+	315.0689	315.0695
	Fragment (loss of CH3N + H2O)		[C17H11ClFN2]+	297.0589	297.0589
	Fragment (loss of C2H5NO)		[C16H11ClFN2]+	285.0588	285.0589
	Fragment (loss of CH3N + C2H3N)		[C15H10ClFNO]+	274.0428	274.0429
	Fragment (loss of C11H10ClN3O)		[C7H6F]+	109.0451	109.0448
	Fragment (loss of C13H9ClFNO)		[C5H7N2]+	95.0608	95.0604
M9	**2 x Oxidation + hydrogenation in methylimidazole**	**3.16**	**C18H15ClFN3O2**	**360.0904**	**360.0910**
	Fragment (loss of C3H5NO2)		[C15H11ClFN2]+	273.0586	273.0589
	Fragment (loss of C6H5F + C3H5NO2)		[C9H6ClN2]+	177.0212	177.0214
	Fragment (loss of C15H10ClFN2)		[C3H6NO2]+	88.0399	88.0393
M10	**2 x Oxidation + Hydrogenation in methylimidazole**	**3.22**	**C18H15ClFN3O2**	**360.0905**	**360.0910**
	Fragment (loss of C2H5NO2)		[C16H11ClFN2]+	285.0584	285.0589
	Fragment (loss of C3H5NO2)		[C15H11ClFN2]+	273.0584	273.0589
	Fragment (loss of C6H5F + C3H5NO2)		[C9H6ClN2]+	177.0212	177.0214
	Fragment (loss of C15H10ClFN2)		[C3H6NO2]+	88.0398	88.0393
M11	**2 x Oxidation + Hydrogenation in methylimidazole**	**3.28**	**C18H15ClFN3O2**	**360.0905**	**360.0910**
	Fragment (loss of C2H5NO2)		[C16H11ClFN2]+	285.0585	285.0589
	Fragment (loss of C3H5NO2)		[C15H11ClFN2]+	273.0587	273.0589
	Fragment (loss of C14H10ClFN2O2)		[C4H6N]+	68.05032	68.0495

M12	**Reductive defluorination**	**3.48**	**C18H14ClN3**	**308.0945**	**308.0949**
	Fragment (loss of NH3)		[C18H12ClN2]+	291.0678	291.0684
	Fragment (loss of Cl)		[C18H15N3]+	273.1253	273.1260
	Fragment (loss of C2H3N)		[C16H12ClN2]+	267.0678	267.0684
	Fragment (loss of C2H3N + CHN)		[C15H11ClN]+	240.0567	240.0575
	Fragment (loss of C2H3N + HCl)		[C16H11N2]+	231.0922	231.0917
	Fragment (loss of C4H6N2)		[C14H9ClN]+	226.0416	226.0418
	Fragment (loss of C4H6N2 + Cl)		[C14H9N]+	191.0728	191.0730
	Fragment (loss of C6H4Cl + C2H3N)		[C10H8N2]+	156.0682	156.0682
M13	**Glucoside conjugation**	**3.11**	**C24H23ClFN3O5**	**488.1379**	**488.1383**
	Fragment (loss of C6H10O5)		[C18H14ClFN3]+	326.0848	326.0855
	Fragment (loss of C6H10O5 + NH3)		[C18H11ClFN2]+	309.0583	309.0589
	Fragment (loss of C6H10O5 + CHN)		[C17H13ClFN2]+	299.0749	299.0746
	Fragment (loss of C6H10O5 + Cl)		[C18H14FN3]+	291.1159	291.1166
	Fragment (loss of C6H10O5 + C2H3N)		[C16H11ClFN2]+	285.0591	285.0589
	Fragment (loss of C6H10O5 + C3H4N2)		[C15H10ClFN]+	258.0481	258.0480
	Fragment (loss of C6H10O5 + C4H6N2)		[C14H8ClFN]+	244.0322	244.0324
	Fragment (loss of C6H10O5 + C6H5F)		[C12H9ClN3]+	230.0477	230.0480
	Fragment (loss of C6H10O5 + C4H6N2 + Cl)		[C14H8FN]+	209.0634	209.0635
	Fragment (loss of C6H10O5 + C7H4FN)		[C11H10ClN2]+	205.0526	205.0527
M14	**Oxidation + hydrogenation + Glucoside conjugation**	**2.80**	**C24H25ClFN3O6**	**506.1491**	**506.1489**

Figure 1. The suggested metabolic pathways for the observed Clozapine metabolites.

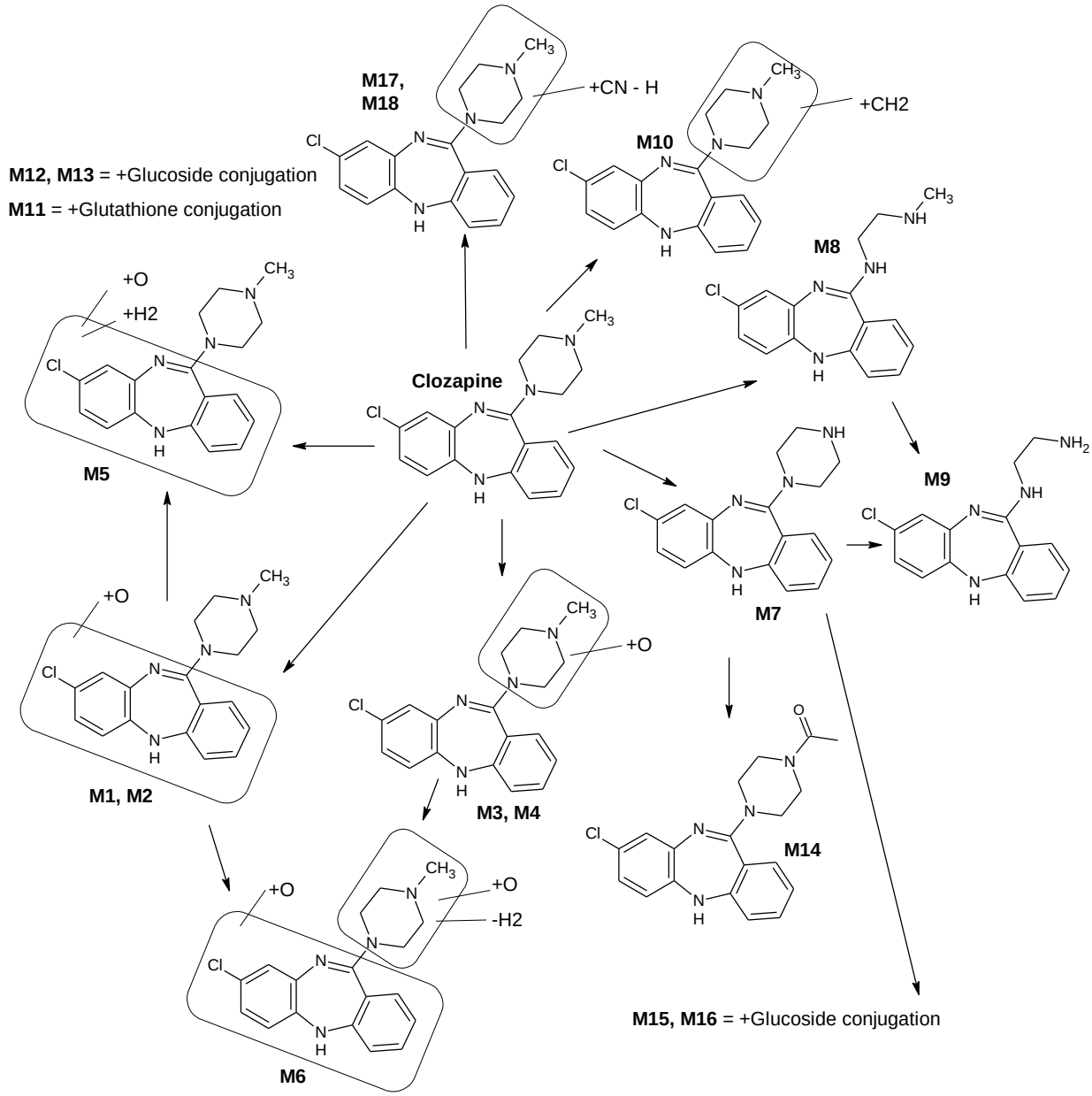
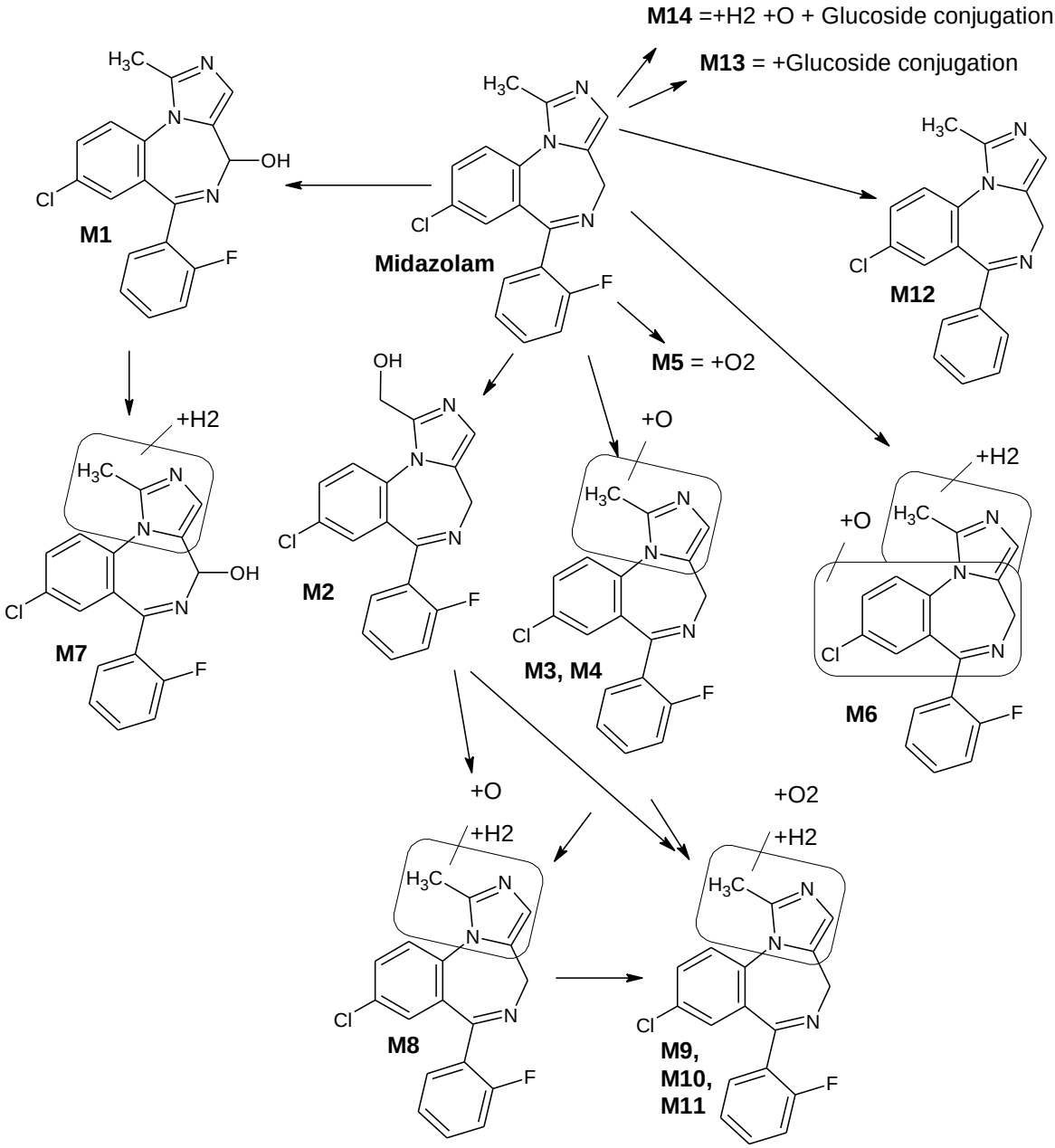


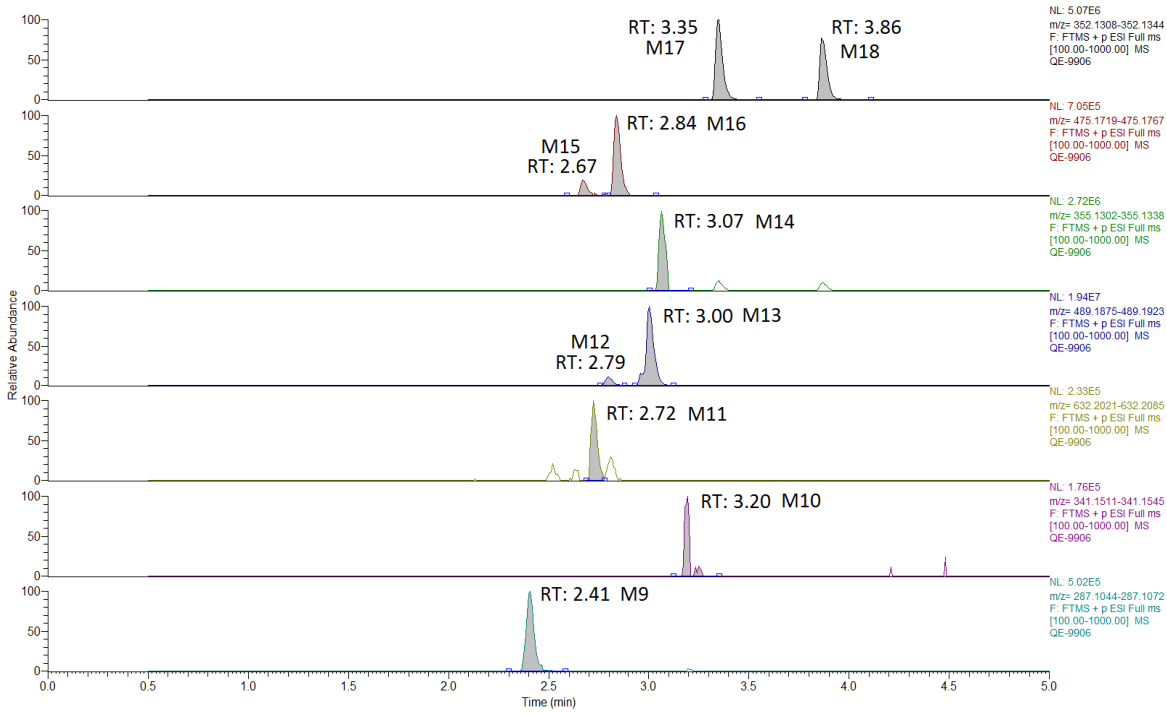
Figure 2. The suggested metabolic pathways for the observed Midazolam metabolites.

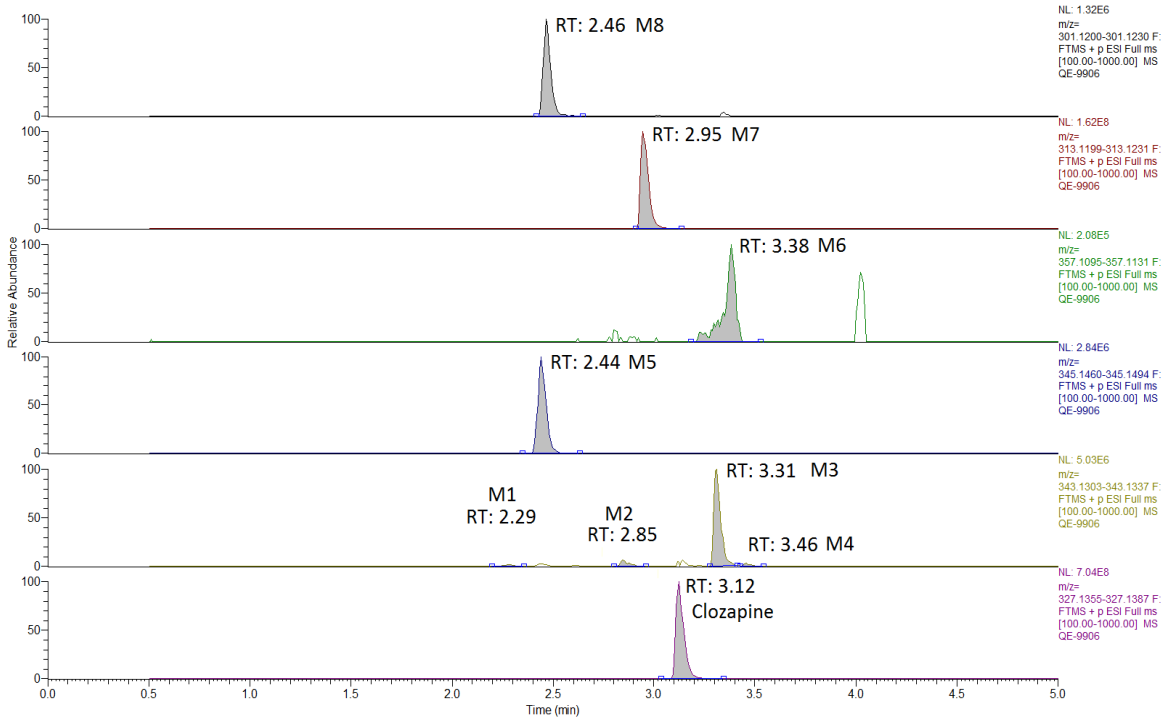


4. APPENDICES

Appendix I LC/Q-TOF-MS ion chromatograms of the Clozapine and its observed metabolites

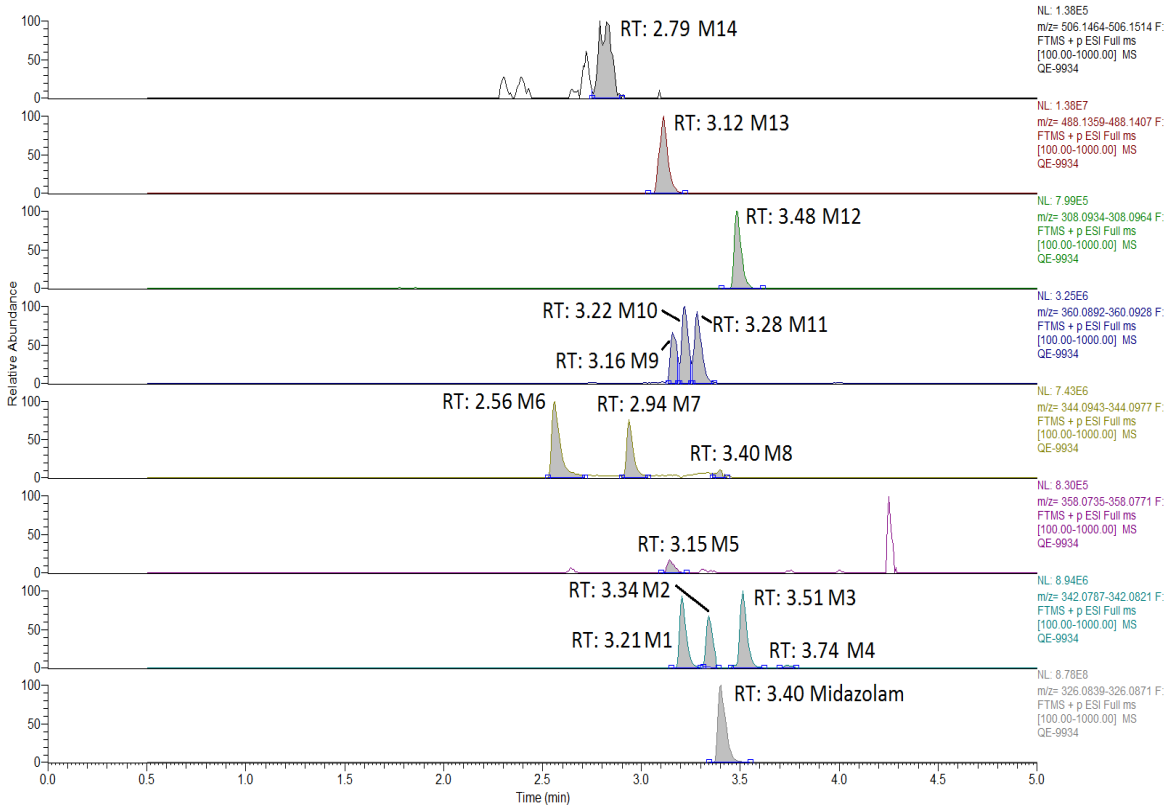
All shown data is from sample nro 15 (10 µM brains 5+6).



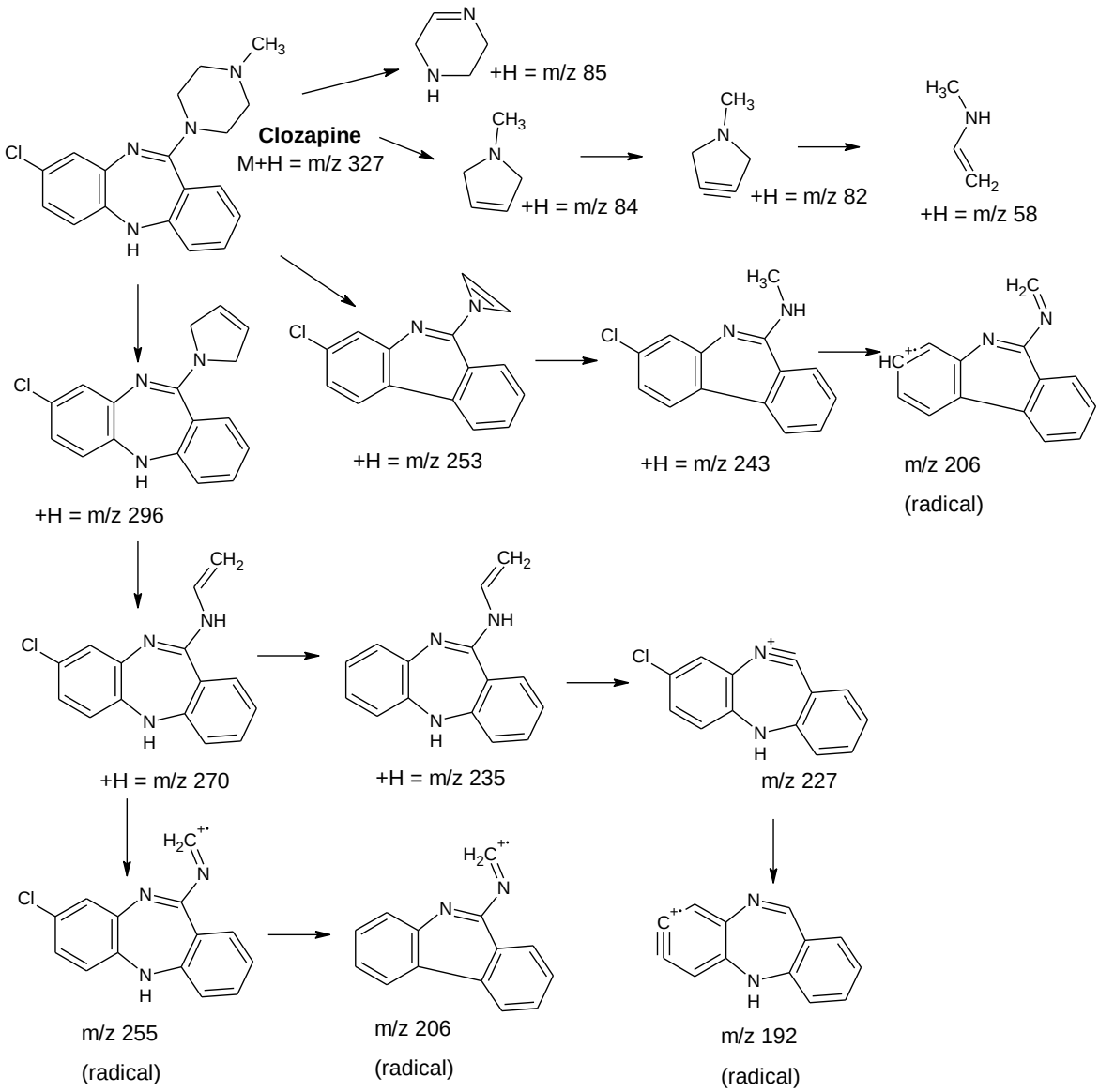


Appendix II LC/Q-TOF-MS ion chromatograms of the Midazolam and its observed metabolites

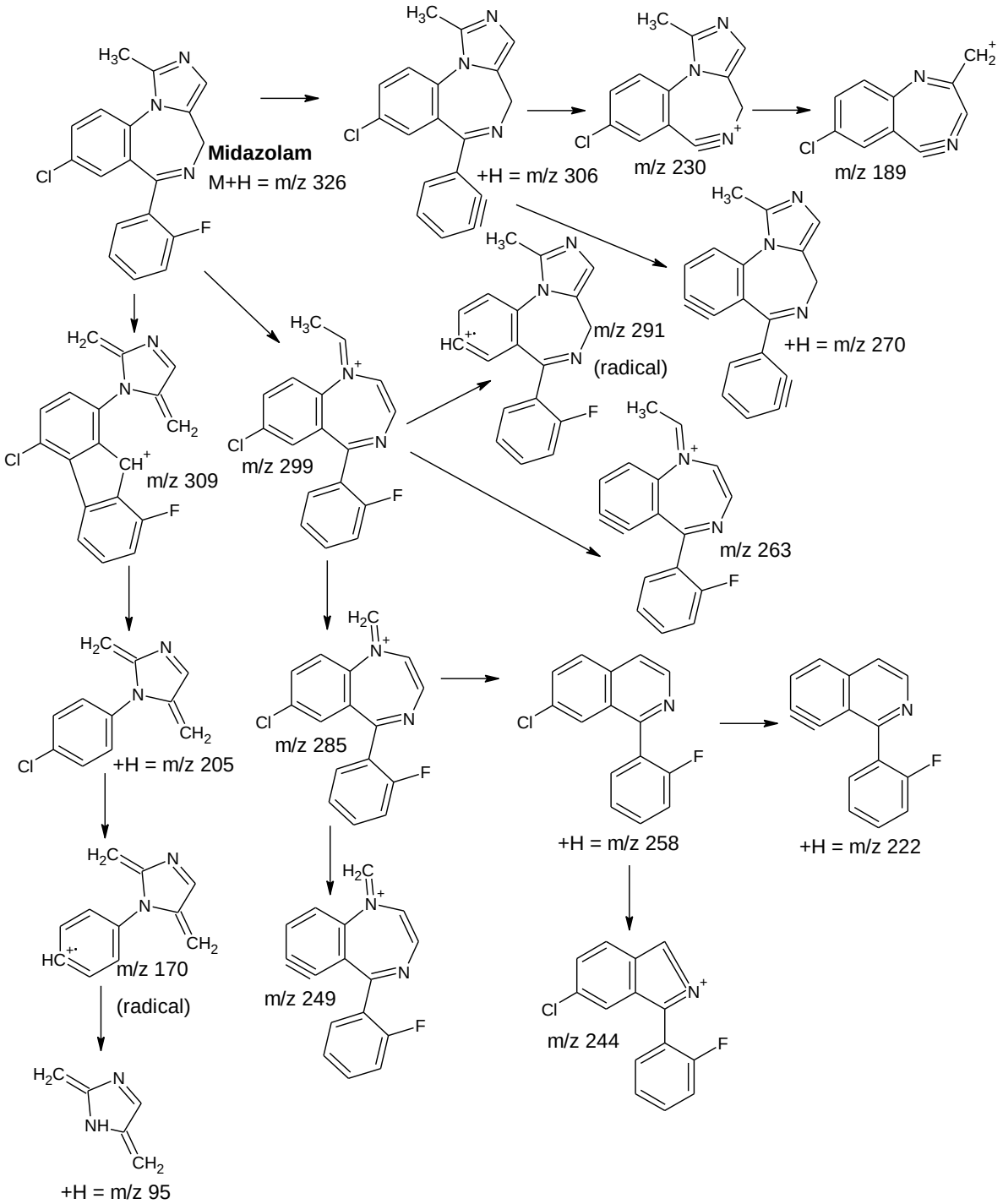
All shown data is from sample nro 33 (10 µM brains 5+6).



Appendix III MS/MS fragment ion identification for Clozapine



Appendix IV MS/MS fragment ion identification for Midazolam



Appendix V Metabolite profile for clozapine in incubations with 1, 3 and 10 µM initial concentration with grasshopper brain. Most abundant metabolites highlighted in bold font.

	% of combined LC/MS peak area in each sample (parent + metabolites = 100%)																		
Sample	**Clozapine**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**	**M15**	**M16**	**M17**	**M18**
1 (1 µM brains 1+2)	**44.2**	-	0.01	0.62	-	0.18	-	**52.6**	0.15	0.17	-	0.01	0.07	**1.58**	0.11	0.02	0.24	0.03	0.40
2 (1 µM brains 3+4)	**42.3**	-	0.004	0.55	-	0.15	-	**54.7**	0.13	0.14	-	0.002	0.09	**1.73**	0.05	0.02	0.09	0.01	0.28
3 (1 µM brains 5+6)	**44.3**	-	0.01	0.92	-	0.20	-	**50.9**	0.14	0.17	-	-	0.21	**2.77**	0.08	0.05	0.20	0.02	0.28
4 (1 µM well 1+2)	**93.6**	-	-	0.50	-	0.12	-	**4.72**	-	-	-	-	-	**1.06**	-	-	-	-	-
5 (1 µM well 3+4)	**95.6**	-	-	0.48	-	0.06	-	**3.41**	-	-	-	-	-	0.49	-	-	-	-	-
6 (1 µM well 5+6)	**94.3**	-	-	0.56	-	0.12	-	**4.20**	-	-	-	-	0.01	0.79	-	-	-	-	-
7 (3 µM brains 1+2)	**63.5**	0.01	0.04	0.87	0.01	0.38	0.01	**32.1**	0.15	0.12	0.01	0.02	0.15	**2.02**	0.31	0.02	0.06	0.23	0.37
8 (3 µM brains 3+4)	**58.5**	0.005	0.03	0.97	0.01	0.36	0.01	**35.2**	0.17	0.16	0.01	0.02	0.19	**2.56**	0.63	0.03	0.12	0.99	0.30
9 (3 µM brains 5+6)	**61.4**	0.01	0.03	0.71	0.01	0.26	0.01	**35.0**	0.17	0.15	0.01	0.01	0.05	**1.84**	0.06	0.01	0.13	0.03	0.39
10 (3 µM well 1+2)	**96.9**	-	-	0.35	-	0.11	-	**2.06**	-	-	0.002		0.02	0.56	-	-	-	-	-
11 (3 µM well 3+4)	**96.0**	-	-	0.40	-	0.14	-	**2.76**	-	-	0.004		0.02	0.67	-	-	-	-	-
12 (3 µM well 5+6)	**95.9**	-	-	0.45	-	0.11	-	**2.90**	-	-	-		0.01	0.65	-	-	-	-	-
13 (10 µM brains 1+2)	**78.2**	0.02	0.04	0.47	0.01	0.36	0.03	**14.7**	0.10	0.03	0.01	0.02	0.59	**4.28**	0.35	0.04	0.09	0.51	0.29
14 (10 µM brains 3+4)	**79.1**	0.02	0.04	0.48	0.02	0.39	0.03	**15.8**	0.13	0.04	0.02	0.02	0.22	**2.67**	0.29	0.01	0.07	0.60	0.37
15 (10 µM brains 5+6)	**77.9**	0.02	0.04	0.50	0.01	0.31	0.03	**17.8**	0.13	0.05	0.01	0.02	0.22	**2.09**	0.24	0.01	0.07	0.49	0.37
16 (10 µM well 1+2)	**97.3**	-	0.00	0.29	-	0.09	-	**1.63**	0.01	-	0.01	0.001	0.05	0.65	-	-	-	-	-
17 (10 µM well 3+4)	**97.2**	-	0.01	0.26	-	0.11	-	**1.72**	0.01	-	0.01	0.005	0.03	0.65	-	-	-	-	-
18 (10 µM well 5+6)	**97.9**	-	0.003	0.26	-	0.08	-	**1.24**	0.01	-	0.01	0.001	0.02	0.45	-	-	-	-	-

Appendix VI Metabolite profile for midazolam in incubations with 1, 3 and 10 µM initial concentration with grasshopper brain. Most abundant metabolites highlighted in bold font.

	% of combined LC/MS peak area in each sample (parent + metabolites = 100%)														
Sample	**Midazolam**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**	**M13**	**M14**
19 (1 µM brains 1+2)	**89.0**	**1.64**	**0.71**	**2.73**	0.10	0.04	**0.40**	**0.65**	0.34	**0.80**	**1.29**	**0.95**	0.04	**1.26**	0.03
20 (1 µM brains 3+4)	**87.4**	**1.29**	**0.61**	**2.95**	0.13	0.03	**0.43**	**0.45**	0.33	**0.94**	**1.54**	**1.11**	0.04	**2.67**	0.08
21 (1 µM brains 5+6)	**89.4**	**1.44**	**0.89**	**2.51**	0.06	0.04	**0.41**	**0.67**	0.28	**0.56**	**0.88**	**0.84**	0.03	**1.96**	0.05
22 (1 µM well 1+2)	**96.9**	0.33	0.06	0.16	0.03	-	0.80	0.20	-	0.09	0.09	0.12	0.01	**1.24**	-
23 (1 µM well 3+4)	**95.3**	0.29	0.06	0.18	0.05	-	0.73	0.22	-	0.14	0.14	0.19	0.01	**2.65**	-
24 (1 µM well 5+6)	**96.9**	0.30	0.06	0.14	0.03	-	0.73	0.22	-	0.05	0.06	0.12	0.02	**1.36**	-
25 (3 µM brains 1+2)	**92.4**	**1.02**	**0.61**	**1.52**	0.06	0.01	**0.48**	**0.42**	0.21	**0.36**	**0.54**	**0.56**	0.05	**1.74**	0.03
26 (3 µM brains 3+4)	**91.2**	**1.19**	**0.79**	**1.04**	0.08	0.02	**0.58**	**0.55**	0.24	**0.28**	**0.46**	**0.52**	0.05	**2.89**	0.07
27 (3 µM brains 5+6)	**91.3**	**1.25**	**0.73**	**1.58**	0.08	0.03	**0.57**	**0.47**	0.26	**0.51**	**0.84**	**0.63**	0.05	**1.64**	0.03
28 (3 µM well 1+2)	**96.9**	0.19	0.04	0.08	0.01	-	0.76	0.19	0.07	0.04	0.04	0.05	0.03	**1.55**	-
29 (3 µM well 3+4)	**97.1**	0.17	0.05	0.08	0.06	-	0.75	0.17	0.10	0.05	0.06	0.13	0.03	**1.28**	-
30 (3 µM well 5+6)	**95.9**	0.30	0.08	0.15	0.07	-	0.73	0.22	0.10	0.07	0.08	0.16	0.04	**2.09**	-
31 (10 µM brains 1+2)	**94.0**	**0.73**	**0.51**	**0.81**	0.04	0.01	**0.79**	**0.45**	0.21	**0.18**	**0.30**	**0.29**	0.07	**1.54**	0.02
32 (10 µM brains 3+4)	**94.3**	**0.65**	**0.50**	**0.79**	0.04	0.01	**0.77**	**0.36**	0.21	**0.18**	**0.27**	**0.28**	0.07	**1.54**	0.03
33 (10 µM brains 5+6)	**94.7**	**0.65**	**0.53**	**0.81**	0.04	0.01	**0.86**	**0.42**	0.22	**0.20**	**0.31**	**0.29**	0.08	**0.84**	0.01
34 (10 µM well 1+2)	**97.9**	0.14	0.04	0.06	0.01	-	0.73	0.13	0.07	0.02	0.02	0.02	0.05	**0.84**	-
35(10 µM well 3+4)	**97.6**	0.14	0.05	0.06	0.01	-	0.83	0.12	0.08	0.03	0.03	0.03	0.05	**0.95**	-
36(10 µM well 5+6)	**97.9**	0.13	0.05	0.05	0.02	-	0.79	0.10	0.09	0.02	0.02	0.03	0.05	**0.71**	-