Supporting Information

Wiseman *et al.* 10.1073/pnas.0801066105

SI Text

Clozapine (Fig. S1a) (Clozaril) is an atypical antipsychotic developed in the 1960s targeted toward treatment of schizophrenia. Dosages in humans are typically between 150–450 mg/day resulting in plasma therapeutic concentrations ranging from 350 to 450 ng/ml (1). The main active metabolites of clozapine are desmethylclozapine (Fig. S1*b*) and *N*-clozapine-*N*-oxide (Fig. S1c). Clozapine distribution in brain, liver, lung, and spleen has been described, including a tendency for slower clearance from lung tissue than brain (2).

1. Perry PJ, Miller DD, Arndt SV, Cadoret RJ (1991) *Am J Psychiatry* 148:231–235.
2. Gardiner T, Lewis J, Shore P (1978) Distribution of clozapine in the rat: Localization in lung. *J Pharmacol Exp Ther* 206:151–157.
3. Han XL, Gross RW (1995) Structural determination of picomole amounts of phospholipids via electrospray ionization tandem mass spectrometry. *J Am Soc Mass Spectrom* 6:1202–1210.
4. Domingues P, Domingues MRM, Amado FML, Ferrer-Correia AJ (2001) Characterization of sodiated glycerol phosphatidylcholine phospholipids by mass spectrometry. *Rapid Commun Mass Spectrom* 15:799–804.

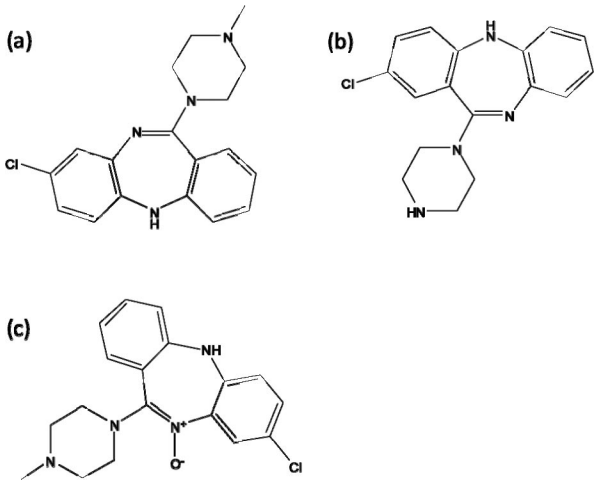

Fig. S1. Chemical structures of (*a*) clozapine, (*b*) *N*-desmethylclozapine, and (*c*) clozapine-*N*-oxide.

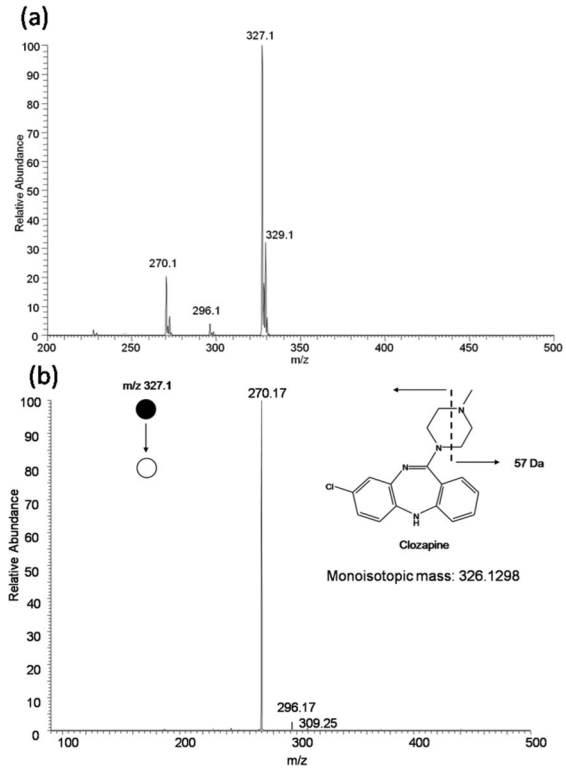

Fig. S2. (*a*) DESI mass spectrum of the authentic clozapine standard. 1 μl of a 10 ng/μl solution of clozapine was deposited onto a PTFE printed glass slide and analyzed with 70:30 methanol:water spray solution under typical DESI conditions. (*b*) DESI MS/MS product ion spectrum of the $M+H^+$ ion at 327.1 (selected by using a 2 *m/z* window) yields a dominant product ion at *m/z* 270.1 by the ring cleavage indicated.

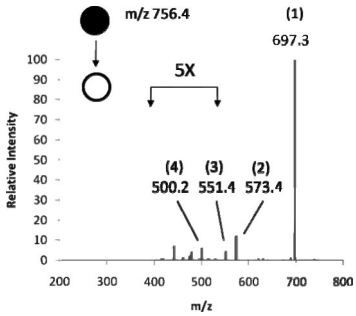

Fig. S3. DESI MS/MS product ion mass spectrum recorded in the positive ion mode by using a Thermo-Fisher LTQ linear ion trap mass spectrometer for the selected ion at *m/z* 756.4 ± 1 detected in lung tissues. Fragmentation of *m/z* 756.4 by using a normalized collision energy of 30 results in a dominant product ion at *m/z* 697.3 (1), corresponding to loss of a neutral fragment of 59 Da assigned as $N(CH_3)_3$ from a phosphatidylcholine (PC) head group. Additional fragments are observed at *m/z* 573.4, *m/z* 551.5, and *m/z* 500.2, corresponding to losses of 183 Da (2), 205 Da (3), and 256 Da (4), respectively. The neutral loss of 59 Da on MS/MS is common to alkylated glycerophosphopholipids $(M+Na/K)^+$ and not the protonated form $(M+H)^+$, because the latter tends to produce a distinct product ion at *m/z* 184, corresponding to the intact PC head group (3, 4). The ions at *m/z* 573.4 and 500.2 are formed by the loss of the PC head group and one of the intact fatty acid chains, respectively. Therefore, we assign this ion as the sodiated form of PC 16:0/16:0 (MW: 733).

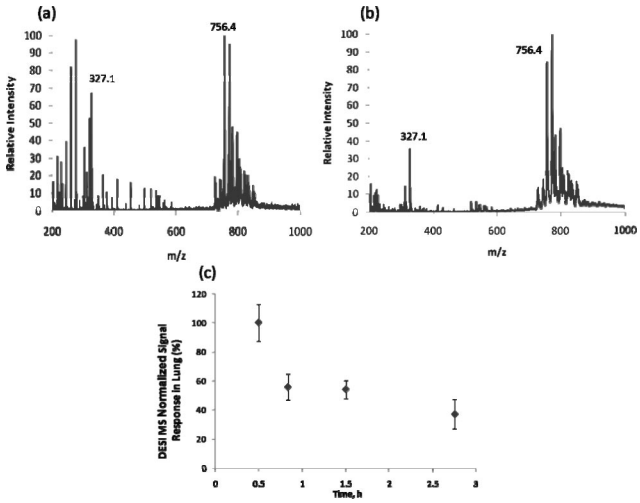

Fig. S4. (*a*) Representative full-scan DESI mass spectrum of rat lung tissue for sample 988. (*b*) Representative full-scan DESI mass spectrum of rat lung tissue for sample 986. (*c*) Normalized signal responses for clozapine in rat lung tissue sections per time-point. The error bars represent the intra-sample precision when three serial sections of each tissue were analyzed.

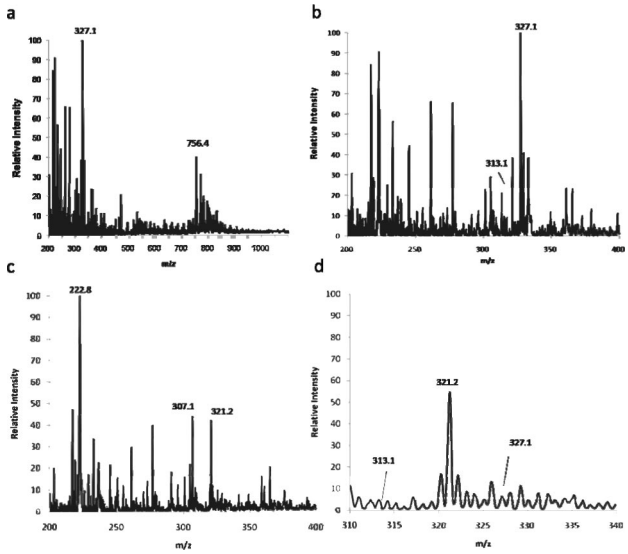

Fig. S5. (*a*) DESI MS mass spectrum of rat lung tissue sample 992. (*b*) DESI MS mass spectrum shown in *a* but over the *m/z* range 200–400. Labeled peaks at 327.1 and 313.1 are assigned to clozapine $(M+H)^+$ and desmethylclozapine $(M+H)^+$, respectively. (*c*) DESI MS mass spectrum of rat lung tissue sample 984 (control) shown over the *m/z* range 200–400. (*d*) DESI MS mass spectrum shown in *c* but over the *m/z* range 310–340 indicating the regions in the baseline which would correspond to clozapine and desmethylclozapine signals in the dosed samples.

Table S1. DESI-MS imaging operating parameters

Parameter	Value
ES voltage	5 KV
Nebulizing gas pressure	100 psi
Solvent flow rate	1.5 μl/min
Impact angle (α)	55°
Tip to surface distance	2 mm
Tip to inlet distance	4 mm