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N-Desalkylquetiapine, a Potent Norepinephrine Reuptake Inhibitor and Partial 5-HT_{1A} Agonist, as a Putative Mediator of Quetiapine's Antidepressant Activity

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Quetiapine is an atypical antipsychotic drug that is also US FDA approved for treating bipolar depression, albeit by an unknown mechanism. To discover the potential mechanism for this apparently unique action, we screened quetiapine, its metabolite *N*-Desalkylquetiapine, and dibenzo[*b*,*f*][1,4]thiazepine-11(10-*H*)-one (DBTO) against a large panel of G-protein–coupled receptors, ion channels, and neurotransmitter transporters. DBTO was inactive at all tested molecular targets. *N*-Desalkylquetiapine had a high affinity (3.4 nM) for the histamine H_1 receptor and moderate affinities (10–100 nM) for the norepinephrine reuptake transporter (NET), the serotonin 5-HT_{1A}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_7 receptors, the α_{1B}-adrenergic receptor, and the M_1, M_3, and M_5 muscarinic receptors. The compound had low affinities (100–1000 nM) for the 5-HT_{1D}, 5-HT_{2C}, 5-HT_3, 5-HT_5, 5-HT_6, α_{1A}, α_{2A}, α_{2B}, α_{2C}, H_2, M_2, M_4, and dopamine D_1, D_2, D_3, and D_4 receptors. *N*-Desalkylquetiapine potently inhibited human NE transporter with a K_i of 12 nM, about 100-fold more potent than quetiapine itself. *N*-Desalkylquetiapine was also 10-fold more potent and more efficacious than quetiapine at the 5-HT_{1A} receptor. *N*-Desalkylquetiapine was an antagonist at 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, α_{1A}, α_{1D}, α_{2A}, α_{2C}, H_1, M_1, M_3, and M_5 receptors. In the mouse tail suspension test, *N*-Desalkylquetiapine displayed potent antidepressant-like activity in VMAT2 heterozygous mice at doses as low as 0.1 mg/kg. These data strongly suggest that the antidepressant activity of quetiapine is mediated, at least in part, by its metabolite *N*-Desalkylquetiapine through NET inhibition and partial 5-HT_{1A} agonism. Possible contributions of this metabolite to the side effects of quetiapine are discussed.

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INTRODUCTION

Quetiapine is an atypical antipsychotic drug that has been widely prescribed since its introduction in 1996 (Gunasekara and Spencer, 1998). Quetiapine apparently differs from other typical and atypical antipsychotic drugs by its antidepressant activity and its proven efficacy in bipolar disorder and depression as demonstrated in several clinical trials (Calabrese *et al*, 2005; Thase *et al*, 2006). The mechanism of this unique antidepressant activity is currently unknown. We have recently described the highly efficacious action of the clozapine metabolite *N*-desmethylclozapine at the muscarinic M_1 receptor and suggested a possible contribution of this activity to the superior clinical efficacy of clozapine. That study highlighted the role that metabolites might play in the actions of antipsychotic drugs (Davies *et al*, 2005). In this regard, quetiapine is extensively metabolized and at least 20 metabolites have been identified (DeVane and Nemeroff, 2001; Goldstein and Arvantis, 1995), although published experimental data on the pharmacological activities of the various metabolites of quetiapine are not available.

The chemical structure of quetiapine and its *N*-desalkyl metabolite (Figure 1) closely resembles several antidepressants, including the secondary amines amoxapine and desipramine. This structural similarity suggests that *N*-Desalkylquetiapine may have antidepressant activity. To

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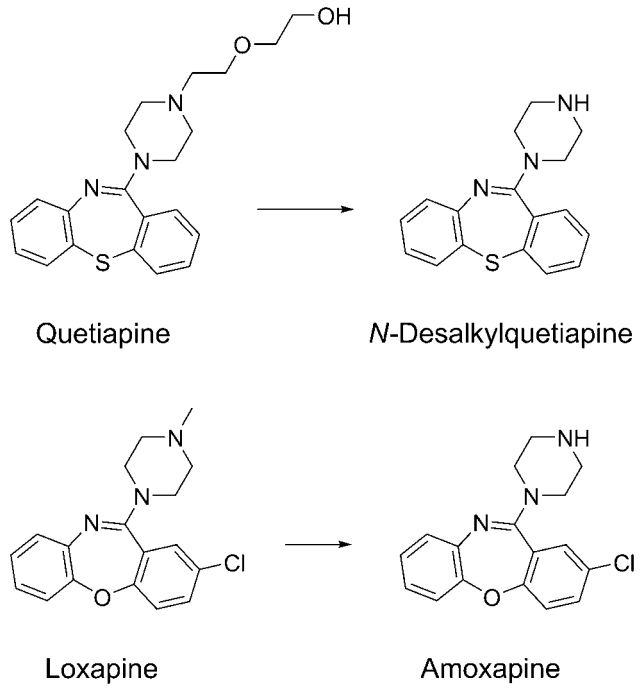

Figure 1 Chemical structures of the atypical antipsychotic drugs quetiapine and loxapine and their metabolites *N*-Desalkylquetiapine and amoxapine. Amoxapine is a widely prescribed antidepressant drug.

elucidate the mechanism of action for quetiapine's unique antidepressant side activity, we have screened quetiapine and *N*-Desalkylquetiapine against a wide range of receptors, ion channels, and neurotransmitter transporters, covering a large subset of the neuro-receptorome (Armbruster and Roth, 2005; Jensen and Roth, 2007). Results from the radioligand-binding screen were validated and further characterized in assays of functional activity. Additionally, *N*-Desalkylquetiapine was tested in the mouse tail suspension test, a model of antidepressant activity, and found to be active.

MATERIALS AND METHODS

Compounds

N-Desalkylquetiapine (11-(piperazin-1-yl)dibenzo[*b*,*f*][1,4] thiazepine) and dibenzo[*b*,*f*][1,4]thiazepin-11(10*H*)-one (DBTO) were purchased from Molcan (Ontario, Canada) and quetiapine was obtained from Sequoia (Pangbourne, UK).

Binding Data

Initial screens and subsequent binding data were obtained using National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP) resources as described earlier (Armbruster and Roth, 2005; Jensen and Roth, 2007; Strachan *et al*, 2006).

GTP-γ-^{35}S Functional Assays

GTP-γ-^{35}S binding was measured in a scintillation proximity assay (SPA) using CHO cells stably expressing the serotonin (5-HT) 5-HT_{1A} receptor and MDCK cells stably expressing the α_{2A} or α_{2C} receptor. Compounds were dissolved in a minimal amount of DMSO in binding buffer and were measured at nine concentrations from 1 to 10 μM in quadruplicate. A dose–response was established for 5-HT or norepinephrine (NE) in each experiment as a control. Nonspecific binding was measured at 10 μM of the compound in the presence of 10 μM WAY-100635 (5-HT_{1A} antagonist) or 32 μM yohimbine (α_2 antagonist) and in the absence of test compound. The background signal was measured at 10 μM test compound in the presence of 10 μM unlabeled GTP-γ-S (Sigma). A premix of 50 μl, prepared from binding buffer (50 mM HEPES, 5 mM $MgCl_2$, 150 mM NaCl, 0.2 mM EDTA, 100 mg/l ascorbic acid, pH 7.4), GDP (Sigma G-7127; 20 μM for 5-HT_{1A} or 2 μM for α_2 assays final), frozen aliquots of cell membranes (1.8–3.5 cm^2 per well), wheat germ agglutinin-coated SPA beads (FlashBlue, PerkinElmer; 100 μg per well), and GTP-γ-^{35}S (PerkinElmer, 1250 μCi/mmol; 300 pM final) was added to 50 μl of the test compounds in flexible transparent PET 96-well plates (PerkinElmer). The sealed plates were incubated for 90 min at room temperature, centrifuged for 5 min at 216*g*, and counted on a Wallac MicroBeta TriLux counter (PerkinElmer). Experiments were replicated at least three times and analyzed using Prism (GraphPad).

Uptake Inhibition Assays

Uptake inhibition at the human NE transporter (hNET), the human dopamine transporter (hDAT), or the human 5-HT transporter (hSERT) was measured using stably transfected HEK 293 cells that were grown in 200 μl per well DMEM containing 5% dialyzed fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 mg/ml streptomycin for 2 days to full confluence at the day of the experiment in tissue culture-treated clear-bottom white opaque 96-well plates that were overcoated with 50 mg/l poly-L-lysine (Sigma, P-1524) in PBS. The medium was changed to serum-free DMEM containing penicillin and streptomycin 16 h before the experiment.

Krebs–Ringer–HEPES (130 mM NaCl, 1.3 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 10 mM HEPES, 1.2 mM KH_2PO_4, 10 mM glucose, 100 mg/l ascorbic acid, pH 7.4) was used as the assay buffer and was supplemented with 100 μM pargyline (MAO inhibitor) and 100 μM tropolone (COMT inhibitor; NET and DAT assays only). NE, dopamine, and 5-HT (all from Sigma) were used as substrates and [^{3}H]NE (49.5 Ci/mmol), [^{3}H]DA (48.1 Ci/mmol), and [^{3}H]5-HT (20.3 Ci/mmol, all from PerkinElmer) were used as radiolabeled substrates. Nisoxetine (Sigma), GBR 12909 (Sigma), and paroxetine (Sequoia) were used as controls and for measuring nonspecific uptake at a concentration of 100 μM. Compounds were dissolved in a minimal amount of DMSO in assay buffer.

Cells were washed with assay buffer at room temperature and were preincubated with 30 μl inhibitor, test compounds, or assay buffer for 10 min at 37°C in a water bath covered with plastic wrap. Radiolabeled substrate (30 μl) was added, the solution was aspirated after 4 min of incubation, and the plates were washed three times with ice-cold buffer. Polystyrene-compatible scintillation cocktail (Microscint PS, PerkinElmer; 50 μl per well) was added and the plate was sealed and agitated for 5 min on an orbital shaker at a

high setting. The plates were counted in a Wallac MicroBeta TriLux counter. Saturation curves were measured in quadruplicate at nine concentrations from 10 to 200 μM of diluted radiolabeled substrate (0.025 μCi per well). Non-specific uptake was measured in the presence of 100 μM inhibitors. K_m values were calculated by fitting against the Michaelis–Menten saturation kinetics model. Uptake inhibition curves were measured in duplicates (standards) or triplicates (test compounds) at 12 concentrations from 0.1 to 100 μM using 1 μM diluted radiolabeled substrate (0.025 μCi per well). IC_{50} values were calculated by fitting the data against a one-site competition model. The apparent K_i values were determined using the Cheng–Prusoff equation. Means ± SEM were calculated from at least three independent experiments.

Uptake inhibition at rat monoamine reuptake transporters was measured as previously described (Rothman *et al*, 2001).

Calcium Flux Assays

HEK 293 cells stably expressing the human H_1, 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptor, rat fibroblast cells stably expressing the human α_{1A} or α_{1D} receptor, and CHO cells stably expressing the human M_1, M_3, or M_5 receptor were incubated for 20 h in serum-free DMEM containing 50 U/ml penicillin and 50 μg/ml streptomycin sulfate in tissue culture-treated black clear-bottom 96- or 384-well plates (Greiner, Germany); plates were overcoated with poly-L-lysine for HEK 293-derived cell lines as described above. The cells were preincubated with 30 μl (96-well plates) or 20 μl (384-well plates) of reconstituted calcium dye (Calcium Plus Assay Kit, Molecular Devices) for 75 min at 37°C in a humidified incubator. The plates were allowed to cool to room temperature for 10 min and were transferred to a FLIPR Tetra fluorescence image plate reader (Molecular Devices). The test compound (30 μl for 96-well plates or 20 μl for 384-well plates) was automatically added and fluorescence was measured for 60 s. In double-addition experiments to detect antagonist activity, the initial response to the test compound was monitored for 15 min (10 min for hH_1 receptor), 30 μl of the endogenous agonist was added, and the second response was monitored for 60 s. The baseline was averaged from the data points immediately before the additions and results were exported as the maximal response over baseline during 60 s after addition. The data were analyzed using Prism (GraphPad).

Animals

The vesicular monoamine transporter 2 (VMAT2) line of 129/C57BL6 mice was developed by deleting transmembrane 3 and 4 regions of the VMAT2 protein (Wang *et al*, 1997). These mice had been backcrossed for 10 generations onto the C57BL/6J genetic background. Wild-type (WT) and heterozygous (HET) mice were obtained by HET breedings (Fukui *et al*, 2007). Male and female mice (2–5 months of age) were used in all experiments. Offspring were weaned after 21 days of age, segregated by sex and genotype, and housed 2–5 mice per cage. Animals were maintained under a 14:10 h light/dark cycle in a humidity- and temperature-controlled room with water and laboratory chow supplied *ad libitum*. All experiments were conducted in accordance with NIH guidelines for the care and use of animals and under approved protocols from the Institutional Animal Care and Use Committee at Duke University.

Tail Suspension Tests

In the single administration experiment, naive WT and VMAT2 HET mice were given vehicle (sterile water; 4 ml/kg) or 0.1, 0.5, or 1.0 mg/kg (i.p.) *N*-Desalkylquetiapine 30 min before testing. In a separate experiment, naive animals were administered vehicle or 0.5 mg/kg *N*-Desalkylquetiapine for 13 days. At the end of that time, they were given the same treatments and tested 30 min later on day 14. Animals were tested in an automated tail suspension apparatus (Med-Associates, St Albans, VT). Time spent in immobility was tabulated at 1 min intervals over 6 min using Med-Associates software.

Open-Field Activity

In the acute study, activities of WT and VMAT2 HET animals were evaluated in the open field 10 days after the tail suspension test. Mice were selected at random (such that animals from each of the injection groups were approximately equally represented) and tail marked with indelible pen and weighed 24 h before testing. Activity was evaluated in the open field in an automated Omnitech Digiscan apparatus (AccuScan Instruments, Columbus, OH) in individual chambers (20 × 20 × 30 cm) with infrared diodes and summed at 5 min intervals over 90 min (Pogorelov *et al*, 2005). Animals were placed into the open field for 30 min, administered vehicle or 1 mg/kg *N*-Desalkylquetiapine (i.p.) and immediately returned to the open field for an additional 60 min. In another experiment, a separate set of mice was given vehicle or 0.5 mg/kg *N*-Desalkylquetiapine for 12 days. Animals were tested on day 13 as described above in the acute study. Locomotion was measured in terms of the total distance traveled (horizontal activity).

Statistics

Behavioral data are reported as means ± SEM and were analyzed with SPSS 11 (SPSS Inc., Chicago, IL). As no sex differences were observed in behavior, data for male and female VMAT2 mice were collapsed and analyzed as a single group. Univariate ANOVA was used to assess genotype differences in the open field for baseline cumulative activity and for cumulative responses to vehicle and *N*-Desalkylquetiapine. ANOVA was used to evaluate effects of vehicle and *N*-Desalkylquetiapine on WT and VMAT2 HET mice in the tail suspension test. *A posteriori* tests were conducted with Bonferroni-corrected pair-wise comparisons, and $p < 0.05$ was considered significant.

RESULTS

Multitarget Screen

The quetiapine metabolite *N*-Desalkylquetiapine and the quetiapine precursor DBTO were subjected to a multitarget binding screen against a large number of G-protein–coupled

receptors, ion channels, and neurotransmitter transporters that are found in the brain (Figure 2, Supplementary Table S1). K_i values were determined for compounds that exhibited greater than 50% inhibition in an initial screen at 10 μM.

N-Desalkylquetiapine had a binding affinity of 3.5 ± 0.48 nM ($n = 2$) for the histamine H_1 receptor, and affinities between 10 and 100 nM for NET and the 5-HT_{1A}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_7, α_{1B}, M_1, M_3, and M_5 receptors. Intermediate affinities between 100 and 1 μM were detected for 5-HT_{1D}, 5-HT_{2C}, 5-HT_3, 5-HT_5, 5-HT_6, α_{1A}, α_{2A}, α_{2B}, α_{2C}, D_1, D_2, D_3, D_4, H_2, M_2, and M_4 receptors. *N*-Desalkylquetiapine had affinities >1 μM at all other tested receptors. The binding affinity for SERT approximated 1 μM and no significant inhibition could be detected at the DAT. DBTO had micromolar binding affinities for the human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors, but lacked significant binding inhibition at 10 μM for all other tested receptors. Quetiapine has been previously screened and published (Kroeze *et al*, 2003; Roth *et al*, 2004); see the open-access NIMH-PDSP Ki Database (http://pdsp.med.unc.edu/). The *N*-Desalkylquetiapine and DBTO screens have not been previously revealed.

Reuptake Transporters

The radioligand-binding data for all three monoamine reuptake transporters were verified and further characterized in functional experiments using HEK 293 cells stably transfected with hNET, hSERT, and hDAT. *N*-Desalkylquetiapine emerged as a potent and selective hNET inhibitor (Table 1; Figure 3) while quetiapine was essentially inactive. A similar profile was also observed at the rat monoamine transporters (Table 2).

5-HT receptors

N-Desalkylquetiapine had a binding affinity of 45 nM at the h5-HT_{1A} receptor. Functional SPA GTP-γ-^{35}S assays using stably transfected CHO cells (Table 3) revealed quetiapine to be a weak 5-HT_{1A} partial agonist. At the 5-HT_7 receptor *N*-Desalkylquetiapine had an affinity of 76 nM. The binding affinities of *N*-Desalkylquetiapine for 5-HT_2-family receptors were 58 (5-HT_{2A}), 14 (5-HT_{2B}), and 110 nM (5-HT_{2C}). Given the high affinity of *N*-Desalkylquetiapine for 5-HT_{2B} receptors and the documented risk of 5-HT_{2B} agonism for valvular heart disease (Roth, 2007; Rothman *et al*, 2000; Setola and Roth, 2003), we examined the functional activity of *N*-Desalkylquetiapine at human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors in a calcium flux assay using stably transfected HEK 293 cells. Neither quetiapine nor its metabolite showed any agonist activity at concentrations as high as 32 μM ($n = 3$–4; data not shown) at any of these receptors.

Histamine Receptors

N-Desalkylquetiapine had a high affinity for the human histamine H_1 receptor of 3.5 nM, a modest affinity of 300 nM for the H_2 receptor, and negligible affinities (>1 μM) for the H_3 and H_4 receptors. In functional assays, *N*-Desalkylquetiapine was an antagonist devoid of agonist activity (Figure 4).

Catecholamine Receptors

N-Desalkylquetiapine had only medium- to low-binding affinity for all tested α-adrenergic sites: 144 nM (α_{1A}), 95 nM (α_{1B}), 240 nM (α_{2A}), 378 nM (α_{2B}), and 740 nM (α_{2C}). *N*-Desalkylquetiapine was devoid of agonist activity at α_{2A}

Table 1 Apparent K_i Values at the Human NET, SERT, and DAT Determined from Functional Uptake Inhibition Experiments (Figure) and Uptake Saturation Curves

	hNET (nM)	hSERT	hDAT
N-Desalkylquetiapine	12 ± 1.4	988 ± 171 nM	>10 μM
Quetiapine	927 ± 233	>10 μM	>10 μM
Nisoxetine	1.1 ± 0.22	ND	ND
Paroxetine	ND	3.3 ± 0.88 nM	ND
GBR 12909	ND	ND	70 ± 29 nM

Abbreviations: hDAT, human dopamine transporter; hNET, human norepinephrine transporter; hSERT, human 5-HT transporter; ND, not determined.
Mean ± SEM of three independent experiments.

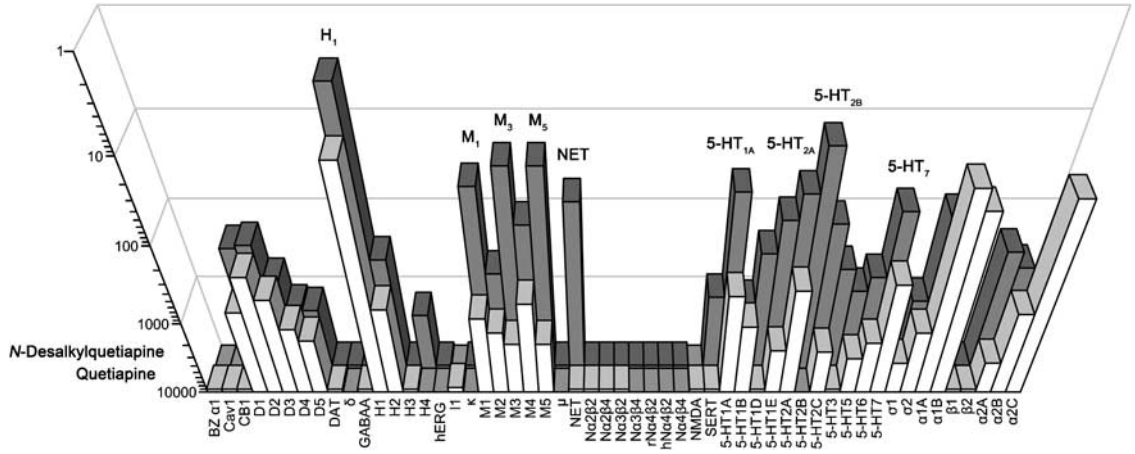

Figure 2 Binding affinities of quetiapine (white bars) and its metabolite *N*-Desalkylquetiapine (dark bars) at the tested panel of brain receptors, ion channels, and neurotransmitter transporters (K_i in nM on a logarithmic scale; no bar, not tested).

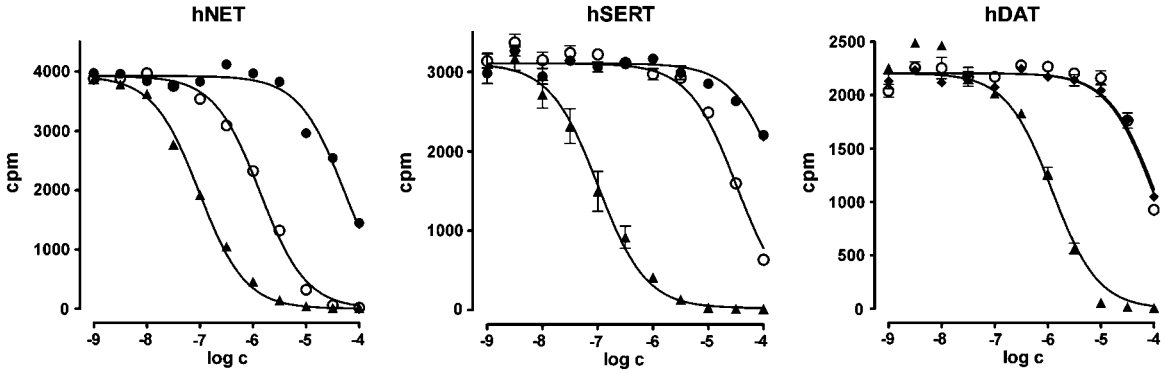

Figure 3 Neurotransmitter reuptake inhibition curves using HEK 293 cells transfected with the human norepinephrine reuptake transporter (NET), 5-HT transporter (SERT), and dopamine transporter (DAT). The transporter-mediated uptake of 1 μM tritium-labeled NE, DA, or 5-HT was measured in the presence of increasing concentrations of *N*-Desalkylquetiapine, quetiapine, or the reuptake inhibitors paroxetine (hNET), GBR 12909 (hDAT), and nisoxetine (hNET). One representative experiment out of three is shown. ○, *N*-Desalkylquetiapine; ●, quetiapine; ▲, paroxetine (hNET); GBR 12909 (hDAT), and nisoxetine (hNET).

Table 2 IC_{50} Values for the Inhibition of the rat NET, SERT, and DAT by *N*-Desalkylquetiapine in Functional Uptake Inhibition Experiments Using Synaptosomal Preparations of Rat Brain Tissue (Rothman *et al*, 2001)

	rNET (nM)	rSERT (nM)	rDAT (nM)
N-Desalkylquetiapine	33 ± 4.4	2359 ± 157	9814 ± 589

Abbreviations: rDAT, rat dopamine transporter; rNET, rat norepinephrine transporter; rSERT, rat 5-HT transporter.
Mean ± SD of three independent experiments.

Table 3 Functional Activity at the Human 5-HT_{1A} Receptor

	5-HT_{1A}	
	EC_{50}	E_{max} (%)
N-Desalkylquetiapine	4898 ± 1043 nM	75 ± 1.6
Quetiapine	> 10 μM	47 ± 12
5-HT	156 ± 49 nM	100
8-OH-DPAT	42 ± 13 nM	56 ± 4.1

GTP-γ-^{35}S binding in the presence of *N*-Desalkylquetiapine, quetiapine, 5-HT, or the partial 5-HT_{1A} receptor agonist 8-OH-DPAT was measured in a scintillation proximity assay (SPA) using CHO cells stably expressing the human 5-HT_{1A} receptor.
Mean ± SEM and the maximal percentage of 5-HT response of three independent experiments.

and α_{2C} receptors in GTP-γ-^{35}S assays ($n = 2$; data not shown) and behaved as an antagonist in calcium flux assays (Figure 4). Negligible binding with affinities above 10 μM was detected at β_1 and β_1 receptors. Low-binding affinities for *N*-Desalkylquetiapine were measured at human DA receptors: 210 nM (D_1), 196 nM (D_2), 570 nM (D_3), 1.3 μM (D_4), and 1.4 μM (D_5). *N*-Desalkylquetiapine at a concentration of 32 μM did not stimulate intracellular cAMP in HEK 293 cells transiently transfected with the D_1 or the D_5 receptor ($n = 3$; data not shown).

Muscarinic Receptors

N-Desalkylquetiapine had medium- to high-affinity for muscarinic receptors: 39 nM (M_1), 453 nM (M_2), 23 nM (M_3), 110 nM (M_4), and 23 nM (M_5). In functional calcium flux assays with M_1, M_3, and M_5 receptors, no agonistic activity was seen up to 32 μM and the compound shifted the acetylcholine dose–response curve rightward ($n = 5$), indicating that *N*-Desalkylquetiapine is an antagonist at M_1, M_3, and M_5 muscarinic receptors (Figure 4).

Tail Suspension Tests

Immobility times were evaluated in the acute experiment following a single injection of vehicle or 0.1, 0.5, or 1.0 mg/kg *N*-Desalkylquetiapine and in the 14-day study following daily administration of vehicle or 0.5 mg/kg *N*-Desalkylquetiapine. VMAT2 HET mice showed increased immobility times under vehicle conditions that were reduced by all tested doses of *N*-Desalkylquetiapine in a dose-dependent manner (Figure 5a). Immobility was also decreased by this compound in animals treated for 14 days (Figure 5b). An ANOVA for the acute treatment revealed main effects of treatment ($F_{3,75} = 20.236$, $p < 0.001$) and genotype ($F_{1,75} = 18.073$, $p < 0.001$), and a significant treatment by genotype interaction ($F_{3,75} = 13.338$, $p < 0.001$). Bonferroni-corrected pair-wise comparisons demonstrated that vehicle-treated VMAT2 HET mice spent more time in immobility than vehicle-treated WT animals ($p < 0.001$). Differences in immobility between genotypes were also found at 0.1 ($p < 0.001$) and 1.0 mg/kg ($p < 0.009$) *N*-Desalkylquetiapine, but not between WT and VMAT2 HET animals treated with the 0.5 mg/kg dose ($p = 0.191$). When compared within genotype, VMAT2 HET animals given 0.1, 0.5, and 1.0 mg/kg *N*-Desalkylquetiapine had reduced immobility times relative to vehicle-treated mutants (p's < 0.008), whereas no effects were found among WT animals (p's > 0.623). Finally, VMAT2 HET animals given 0.5 mg/kg *N*-Desalkylquetiapine did not differ from WT animals administered vehicle ($p > 0.253$), although immobility times for HET animals given 0.01 mg/kg ($p < 0.032$) were higher and mutants

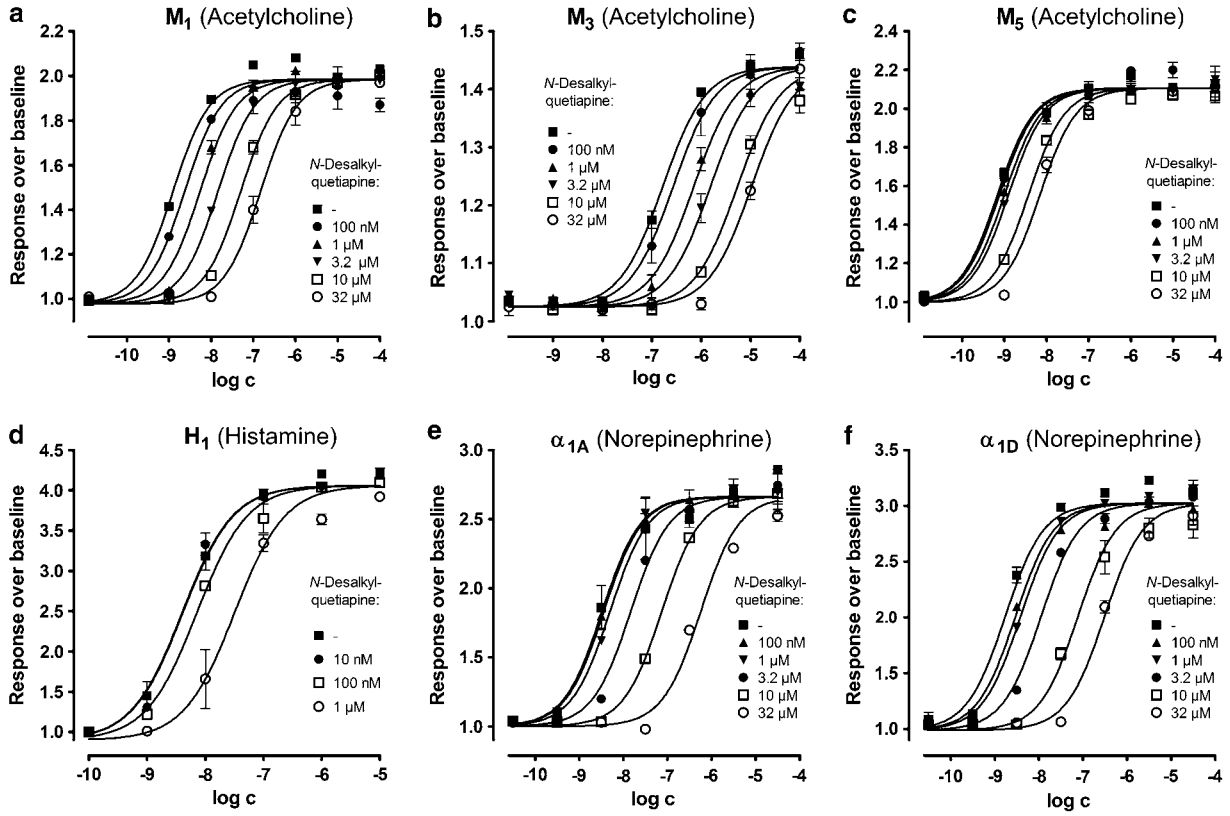

Figure 4 *N*-Desalkylquetiapine acts as an antagonist at the human M_1 (a), M_3 (b), and M_5 (c) muscarinic, the H_1 histaminic (d), and at the α_{1A}- (e) and α_{1D}-adrenergic (f) receptors. Stable cell lines were stimulated with acetylcholine (M_1, M_3, and M_5), histamine (H_1), or norepinephrine (α_{1A} and α_{1D}) in the presence of increasing concentrations of quetiapine and intracellular calcium release was measured in calcium flux assays using a fluorescent dye. *N*-Desalkylquetiapine shifts the dose–response curves of all endogenous ligands rightward, indicating that it is an antagonist. Shown are representative experiments.

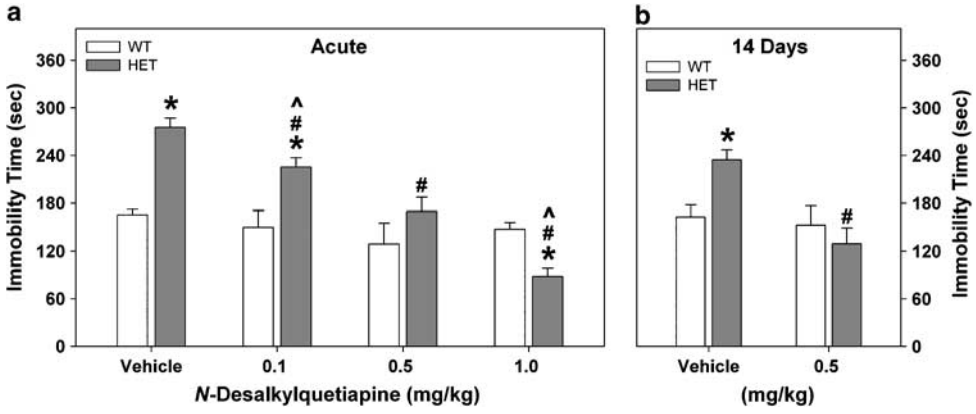

Figure 5 Immobility time during tail suspension testing for wild-type (WT; white bars) and VMAT2 heterozygous (HET; dark bars) animals. (a) Responses following 30 min after acute injection of vehicle or 0.1, 0.5, or 1.0 mg/kg *N*-Desalkylquetiapine. (b) Immobility time 30 min after administration of vehicle or 0.5 mg/kg *N*-Desalkylquetiapine that had been given daily for 14 days. Immobility time was aggregated over the entire 6 min test. $^{*}p<0.05$, WT compared to VMAT2 HET animals; $^{\#}p<0.05$, within genotype comparisons of 0.1, 0.5 or 1.0 mg/kg *N*-Desalkylquetiapine to vehicle treatment; $\hat{}\,p<0.05$, VMAT2 HET treated with *N*-Desalkylquetiapine compared to vehicle-treated WT mice. All data are shown as mean ± SEM with $n=8$–13 mice per genotype per treatment.

administered 1 mg/kg *N*-Desalkylquetiapine were lower ($p<0.023$) than WT controls.

ANOVA for the 14-day experiment failed to reveal an overall effect for genotype ($F_{1,36}=1.600$, $p>0.214$), however, treatment effects ($F_{1,36}=9.302$, $p<0.004$) and the genotype by treatment interaction ($F_{1,36}=6.310$, $p<0.017$) were significant. Bonferroni-corrected pair-wise comparisons showed that WT mice given vehicle spent less time in immobility than VMAT2 HET animals ($p<0.011$). Fourteen days of *N*-Desalkylquetiapine treatment did not alter WT responses relative to their vehicle controls ($p>0.691$), whereas this treatment significantly reduced immobility

times in VMAT2 HET mice ($p<0.001$). Notably, mutants given the compound did not differ from similarly treated WT ($p>0.384$) or WT vehicle controls ($p>0.377$). Together, these data demonstrate that VMAT2 HET mice display depressive-like behavior in the tail suspension test, and that *N*-Desalkylquetiapine given acutely or over 14 days can alleviate this response. Moreover, VMAT2 HET animals are much more sensitive to the immobility-reducing effects of *N*-Desalkylquetiapine than similarly treated WT littermates.

Locomotor Activity

When placed into the open field for 30 min, WT and VMAT2 HET mice showed no differences in basal locomotor activity regardless of dosing assignment. Baseline open-field activity was examined across the different treatment groups (ie vehicle and *N*-Desalkylquetiapine, acute and 14-day administration). ANOVA failed to find main effects of genotype ($F_{1,49}=1.240$, $p>0.271$) or treatment ($F_{2,49}=2.785$, $p>0.072$), or the genotype by treatment interaction ($F_{2,49}=0.043$, $p>0.978$) to be significant. Since baseline activity was not affected, effects of *N*-Desalkylquetiapine and vehicle were examined in the acute and 14-day experiments. Again, main effects of genotype ($F_{1,49}=0.329$, $p>0.561$) and treatment ($F_{2,49}=2.409$, $p>0.093$), and the genotype by treatment interaction ($F_{2,49}=0.158$, $p>0.855$) were not significant. These analyses demonstrate that neither acute nor 14 days of administration of *N*-Desalkylquetiapine exerted any effects on locomotor activity of WT or VMAT2 HET animals. Hence, the effects of quetiapine metabolite in the tail suspension test for VMAT2 HET mice can be attributed to specific reductions in immobility and cannot be attributed to any overall changes in activity for these mutants.

DISCUSSION

The main finding of this paper is that *N*-Desalkylquetiapine is a potent hNET inhibitor and that it possesses antidepressant activity in the tail suspension test for VMAT2 HET mice *in vivo*. Our results imply that the antidepressant effects of quetiapine in bipolar depression (Calabrese *et al*, 2005; Thase *et al*, 2006) are mediated, at least in part, by the potent antidepressant activity of *N*-Desalkylquetiapine. These results underscore the importance of obtaining comprehensive pharmacological profiles of medications and their main metabolites as a means of illuminating their actions *in vivo*. For example, drug-induced valvular heart disease is frequently due to the 5-HT_{2B} receptor agonism of metabolites (Roth, 2007; Rothman *et al*, 2000), while the M_1 muscarinic receptor activation by the metabolite desmethylclozapine may contribute to the clinical efficacy of the antipsychotic drug clozapine (Davies *et al*, 2005). To this list, we can now add the apparent antidepressant actions of *N*-Desalkylquetiapine due, perhaps, to potent inhibition of hNET. Not surprisingly, the US FDA has issued a draft guidance related to safety testing of drug metabolites (http://www.fda.gov/cder/guidance/6366dft.htm).

Quetiapine is extensively metabolized in the body. At least 20 metabolites have been identified, but comprehensive data regarding their plasma concentrations in humans have not yet been made public (DeVane and Nemeroff, 2001; Goldstein and Arvantis, 1995). Phase I reactions include oxidation of the terminal hydroxy group to the carboxylic acid, oxidation of the dibenzothiazepine sulfur to the sulfoxide, aromatic hydroxylation, especially in position 7, and cleavage of the amino side chain at the ether oxygen and the nitrogen (Figure 6). *In vitro* tests using liver microsomes and studies in patients using cytochrome inhibitors and inducers suggest the involvement of cytochrome oxidases, particularly the CYP43A isozyme (Grimm *et al*, 2006). Quetiapine plasma concentrations do not correspond well with receptor occupation levels and clinical effects (Mauri *et al*, 2007), suggesting the involvement of active metabolites in quetiapine's clinical actions. Published experimental data on the pharmacological activities of the many metabolites of quetiapine are not available, although 7-hydroxy-quetiapine and 7-hydroxy-*N*-Desalkylquetiapine have been reported as 'active metabolites' (Davis *et al*, 1999; Gefvert *et al*, 1998).

We have previously noted the structural similarity of the *N*-Desalkylated metabolite of quetiapine (Figure 1) with tricyclic antidepressant drugs like amoxapine and have hypothesized its involvement in quetiapine's clinical profile. The involvement of a metabolite in quetiapine's antidepressant action in humans was also suggested by published animal studies showing antidepressant-like activity after chronic, but not acute, administration (Orsetti *et al*, 2007; Yan *et al*, 2007).

The *N*-Desalkylquetiapine metabolite of quetiapine became commercially available recently and allowed us to test our hypothesis. We subjected the compound to a multitarget screen against a large panel of brain receptors, transporters, and channels and have verified the results in functional assays and in mice using an animal model of antidepressant activity.

Antidepressant Activity

Tricyclic antidepressant drugs exert their action by blocking the reuptake of NE and, to a lesser extent, 5-HT into synaptic terminals. Recent observations indicate that the

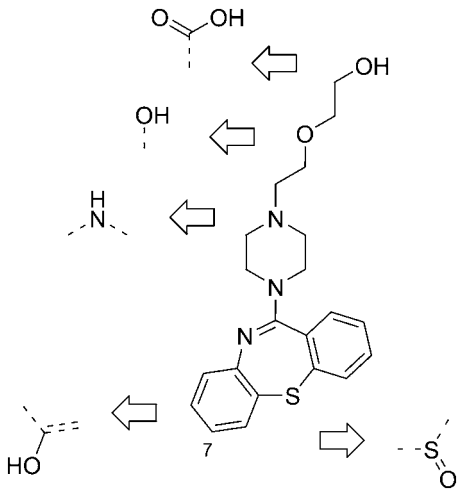

Figure 6 Reported phase I metabolic reactions of quetiapine. Subsequent reactions give rise to a large number of possible metabolites for which no pharmacological data have been published.

use of adjunctive, standard antidepressant medication in the treatment of bipolar depression, as compared with the use of mood stabilizers, such as lithium or valproate alone, does not increase antidepressant efficacy (Sachs *et al*, 2007). However, the adjunctive use of the selective serotonin reuptake inhibitor fluoxetine with the atypical antipsychotic, olanzapine does increase efficacy as compared to olanzapine alone (Tohen *et al*, 2003).

As hypothesized, the quetiapine metabolite *N*-Desalkylquetiapine had high affinity for NET and negligible affinities for SERT and DAT in the initial screen. To verify the functional significance of the radioligand displacement data, we tested the metabolite and its parent compound quetiapine in living HEK 293 cells expressing the human monoamine transporters. These experiments demonstrated a potent and functional inhibition of hNET by the metabolite with an apparent K_i of 12 nM and an about 100-fold selectivity over hSERT and a more than 1000-fold selectivity over hDAT (Table 1). The parent compound quetiapine had no significant binding affinity for hNET and was about two orders of magnitude less potent in the functional assay (Figure 2).

At the 5-HT_{1A} receptor, *N*-Desalkylquetiapine was about 10-fold more potent in binding assays than its parent compound quetiapine (45 *vs* 430 nM). In functional GTP-γ-^{35}S assays the metabolite was also more efficacious than quetiapine (75 *vs* 47% of the maximal 5-HT response). Similar to previously reported data, the EC_{50} values from GTP-γ-^{35}S assays in the current study were about 100-fold higher than the K_i values from binding assays that use the partial agonist [^{3}H]8-OH-DPAT as radioligand (Alper and Nelson, 1998). This is probably related to a predominantly G-protein–uncoupled pool of receptors under the conditions of the functional assay. *N*-Desalkylquetiapine has a binding affinity similar to common 5-HT_{1A} agonist medications like buspirone and gepirone (Becker *et al*, 2006).

Serotonergic and noradrenergic pathways are assumed to independently and in parallel mediate antidepressant activity (Dranovsky and Hen, 2006), and dual action SERT/NET inhibitors like mirtazapine, venlafaxine, and duloxetine have been reported to reduce the symptoms of depression more effectively than selective 5-HT reuptake inhibitors alone (Jain, 2004; Roth *et al*, 2004). The 5-HT_{1A} receptor has been implicated in the mechanism of antidepressant drugs and 5-HT_{1A} (partial) agonists are clinically effective antidepressants and anxiolytics (Becker *et al*, 2006). It is therefore likely that the 5-HT_{1A} receptor partial agonism of the metabolite, in combination with NET inhibition, plays a role in the antidepressant activity of quetiapine.

Because the potent reuptake inhibition of *N*-Desalkylquetiapine at NET as well as its 5-HT_{1A} receptor agonism strongly suggested an antidepressant activity, we tested the compound *in vivo* in the mouse tail suspension test for antidepressant-like activity (Cryan *et al*, 2005). VMAT2 HET mice display depressive-like behaviors in the absence of anxiety-like responses and are responsive to fluoxetine, reboxetine, and bupropion in this test (Fukui *et al*, 2007). As expected from the *in vitro* data, *N*-Desalkylquetiapine demonstrated antidepressant-like activity at doses as low as 0.1 mg/kg without affecting locomotor activity, suggesting a bona fide antidepressant action.

N-Desalkylquetiapine has also a higher affinity for the 5-HT_7 receptor (76 nM) compared to quetiapine (307 nM). The 5-HT_7 receptor has been implicated in depression and circadian sleep disorder, 5-HT_7 receptor knockout mice show a significant decrease in immobility in the forced swim test (a test for antidepressant-like activity), and 5-HT_7 antagonists have the same effects in WT mice when tested during the dark phase of the cycle (Guscott *et al*, 2005). Human data on the effects of 5-HT_7 antagonists are not yet available, but it is possible that 5-HT_7 receptor-mediated effects contribute to the antidepressant-like action of the metabolite and the antidepressant effects of quetiapine pharmacotherapy.

N-Dealkylation of quetiapine did not significantly affect its moderate binding affinities at DA receptors, suggesting that *N*-Desalkylquetiapine might undergo further metabolic activation to yield a possibly more potent DA antagonist such as its 7-hydroxy analog.

Critical Off-Target Activities

Combining a multitarget screening approach with statistical analysis of large data sets has allowed us to predict that histamine H_1 receptors and, to a lesser extent, 5-HT_{2C} receptors, are responsible for antipsychotic drug-induced weight gain (Kroeze *et al*, 2003). Our findings have recently been confirmed in other studies (Silvestre and Prous, 2005), and in H_1 receptor knockout mice the mechanism has been traced to H_1-mediated hypothalamic AMP kinase activation (Kim *et al*, 2007). Quetiapine is a histamine H_1 receptor antagonist with a K_i of 11 nM, which induces weight gain (Allison *et al*, 1999) and has pronounced sedative effects (Cohrs *et al*, 2004; Thase *et al*, 2006). The current results show that its metabolite *N*-Desalkylquetiapine is a very potent H_1 antagonist with an affinity of 3.5 nM and is thus predicted to have weight gain liability. Clearly, however, these predictions are considered tentative in the absence of clinical trial data with *N*-Desalkylquetiapine.

Since *N*-Desalkylquetiapine has a sixfold higher affinity for the 5-HT_{2A} receptor compared to quetiapine, it is possible that this metabolite contributes to the antipsychotic activity of quetiapine—given the prominent role of 5-HT_{2A} receptors in antipsychotic drug actions (Meltzer *et al*, 2003; Roth, 2006). Quetiapine is often prescribed off-label for treating sleep disorders and it is interesting to note that 5-HT_{2A} antagonists have been proposed for use in treating insomnia. It is conceivable that the 5-HT_{2A} antagonism of *N*-Desalkylquetiapine, in addition to its histamine H_1 antagonism, contributes to the marked sedative effects observed during quetiapine treatment.

It has been shown by us and others that drugs that activate the 5-HT_{2B} receptor, often through their metabolites, can cause serious valvular heart disease (Rothman *et al*, 2000) and several medications have already been withdrawn from the market due to this side effect (Roth, 2007). It was therefore of significant clinical importance to test the quetiapine metabolite in functional assays for agonistic action at the 5-HT_{2B} receptor. In functional assay, the *N*-Desalkylquetiapine as well as quetiapine acted as pure antagonists at 5-HT_{2A} and 5-HT_{2B} receptors. These results suggest that neither quetiapine nor *N*-Desalkylquetiapine are likely to cause valvulopathic side

effects, although given the large number of other potential metabolites (Figure 6) we cannot exclude the possibility that other metabolites might emerge as potent 5-HT_{2B} agonists.

N-Desalkylquetiapine is a potent ligand with low-nanomolar affinity for the G_q-coupled muscarinic M_1, M_3, and M_5 receptors. In contrast to the structurally related clozapine metabolite *N*-desmethylclozapine, a highly efficacious M_1 agonist (Davies *et al*, 2005), *N*-Desalkylquetiapine is a pure antagonist at these receptors. The quetiapine metabolite has a 20- to 80-fold increased affinity for M_1, M_3, and M_5 receptors compared to its parent compound. This metabolic activation is of potential clinical significance as M_3 antagonism has been postulated as a contributing factor for antipsychotic-induced diabetes and hyperglycemia (Johnson *et al*, 2005; Silvestre and Prous, 2005). Moreover, antimuscarinic activity is commonly associated with side effects, such as dry mouth, mydriasis, increased intraocular pressure, urinary retention, and hyperthermia. Most of these adverse effects have indeed been observed after overdosing quetiapine (Balit *et al*, 2003) and occasionally during quetiapine treatment, and *N*-Desalkylquetiapine possibly contributes to these effects.

N-Desalkylquetiapine has a strong structural similarity to the class of the tricyclic antidepressants. Tricyclic antidepressants are known for their ability to cause sudden cardiac death in higher chronic dosages or, especially, in case of accidental or suicidal overdoses (Ray *et al*, 2004). This side effect is caused by inhibition of the hERG potassium channel and subsequent prolongation of the QT interval (Recanatini *et al*, 2005; Song and Clark, 2006). However, no hERG inhibition up to 10 μM could be detected for *N*-Desalkylquetiapine in the screening, suggesting that *N*-Desalkylquetiapine might be free of cardiovascular liability.

In conclusion, our data imply that the antidepressant activity of quetiapine is mediated, at least in part, by its metabolite *N*-Desalkylquetiapine through a selective NE reuptake inhibition and 5-HT_{1A} and 5-HT_7 receptor activities. Obviously, because no data are available on the plasma concentrations of *N*-Desalkylquetiapine, we can only infer that the antidepressant actions of quetiapine are mediated by this metabolite. Clearly, clinical studies are needed to support this notion. Clinical side effects associated with its potent histamine H_1 antagonism and antimuscarinic activity are predicted based on its *in vitro* pharmacology. The current work further demonstrates the need to screen medications as well as all their metabolites for side activities against a wide panel of pharmacologically relevant targets.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare that over the past 3 years they have received financial compensation from sources other than their primary employer as follows:

Dr Rodriguiz has received compensation from Med-Associates as a workshop teacher.

Dr Caron's work has been funded by the NIH. He has received compensation as a member of the scientific advisory board of Acadia Pharmaceutical and owns stock in the company. He also has consulted for Lundbeck and has received compensation. He has also received compensation in the form of honoraria for lecturing at various scientific meetings and academic institutions.

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Dr Rothman and Dr Jensen declare no potential conflict of interest and have no financial compensation to disclose.

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