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RESEARCH PAPER

Quetiapine and its metabolite norquetiapine: translation from *in vitro* pharmacology to *in vivo* efficacy in rodent models

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BACKGROUND AND PURPOSE

Quetiapine has a range of clinical activity distinct from other atypical antipsychotic drugs, demonstrating efficacy as monotherapy in bipolar depression, major depressive disorder and generalized anxiety disorder. The neuropharmacological mechanisms underlying this clinical profile are not completely understood; however, the major active metabolite, norquetiapine, has been shown to have a distinct *in vitro* pharmacological profile consistent with a broad therapeutic range and may contribute to the clinical profile of quetiapine.

EXPERIMENTAL APPROACH

We evaluated quetiapine and norquetiapine, using *in vitro* binding and functional assays of targets known to be associated with antidepressant and anxiolytic drug actions and compared these activities with a representative range of established antipsychotics and antidepressants. To determine how the *in vitro* pharmacological properties translate into *in vivo* activity, we used preclinical animal models with translational relevance to established antidepressant-like and anxiolytic-like drug action.

KEY RESULTS

Norquetiapine had equivalent activity to established antidepressants at the noradrenaline transporter (NET), while quetiapine was inactive. Norquetiapine was active in the mouse forced swimming and rat learned helplessness tests. In *in vivo* receptor occupancy studies, norquetiapine had significant occupancy at NET at behaviourally relevant doses. Both quetiapine and norquetiapine were agonists at 5-HT_{1A} receptors, and the anxiolytic-like activity of norquetiapine in rat punished responding was blocked by the 5-HT_{1A} antagonist, WAY100635.

CONCLUSIONS AND IMPLICATIONS

Quetiapine and norquetiapine have multiple *in vitro* pharmacological actions, and results from preclinical studies suggest that activity at NET and 5-HT_{1A} receptors contributes to the antidepressant and anxiolytic effects in patients treated with quetiapine.

Abbreviations

DAT, dopamine transporter; E_{max}, maximal concentration; FST, forced swimming test; LH, learned helplessness; NET, noradrenaline transporter; SERT, 5-HT transporter; SPA, scintillation proximity assay

Tables of Links

TARGETS	
GPCRs[a]	**Transporters**[c]
5-HT_{1A} receptor	DAT
5-HT_{2A} receptor	NET
5-HT_{2C} receptor	SERT
Ligand-gated ion channels[b]	
AMPA receptors	
Kainate receptors	
NMDA receptors	

LIGANDS		
8-OH-DPAT	Duloxetine	Quetiapine
[^{3}H]-AMPA	Escitalopram	Raclopride
[^{3}H]-CGP39653	GTPγS	Reboxetine
[^{3}H]-mesulergine	Imipramine	Risperidone
Aripiprazole	MDL100907	Sertraline
Atomoxetine	Mianserin	WAY100635
Buspirone	Nisoxetine	WIN 35428
Clozapine	Norquetiapine	Ziprasidone
Desipramine	Olanzapine	

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

Quetiapine was originally developed as a second-generation antipsychotic agent for the treatment of schizophrenia; however, the compound demonstrates a broad range of clinical activity across several neuropsychiatric disorders. Similar to several second-generation antipsychotic drugs, quetiapine has been shown to be an effective treatment for psychiatric disorders including schizophrenia and bipolar mania and as an adjunct treatment of depression (e.g. McIntyre *et al.*, 2005; Hirschfeld *et al.*, 2006; Thase *et al.*, 2006; Bauer *et al.*, 2009). However, quetiapine has also demonstrated efficacy in large clinical trial programmes as a monotherapy in bipolar depression (Calabrese *et al.*, 2005), major depressive disorder (Cutler *et al.*, 2009; Weisler *et al.*, 2009) and generalized anxiety disorder (Bandelow *et al.*, 2010). This combination of anxiolytic and antidepressant efficacy was not originally predicted based on the preclinical pharmacology of quetiapine and would not be expected of a drug often described within the broad term: 'atypical antipsychotic'. Indeed, amongst the antipsychotic drugs, quetiapine alone has shown robust efficacy as monotherapy in major depressive disorder and generalized anxiety disorder clinical trials (Komossa *et al.*, 2010).

Several recent findings have generated potential explanations for the antidepressant effects of quetiapine seen in clinical trials. Firstly, *N*-desalkyl quetiapine (or norquetiapine) was identified as a major active metabolite of quetiapine in humans (Winter *et al.*, 2008), and secondly, it was noted that norquetiapine has a distinct *in vitro* pharmacological profile compared with quetiapine and other atypical antipsychotic drugs. Thus, within the range of targets screened *in vitro*, norquetiapine inhibits the noradrenaline transporter (NET) and has partial agonist activity at 5-HT_{1A} receptors (Goldstein *et al.*, 2007; Jensen *et al.*, 2008). Activity at both these targets is hypothesized to contribute to antidepressant activity. It should be noted that differences have also been observed in the activity of quetiapine and norquetiapine at 5-HT_2 receptors, which may also contribute to the clinical profile.

While receptor binding and functional profiles for quetiapine and norquetiapine have been studied within a screening paradigm, the translation in relation to mechanisms of antidepressant and anxiolytic effects has not been systematically studied either in animal models or in human translational pharmacology.

In the present study, we further characterized the *in vitro* binding and *in vitro* pharmacological properties of quetiapine and norquetiapine at targets known to be associated with antidepressant and anxiolytic drug action and compared these activities with a representative range of established antipsychotics and antidepressants. In addition, we examined how the *in vitro* pharmacological properties of quetiapine and norquetiapine translate into *in vivo* activity in preclinical animal models of antidepressant-like and anxiolytic-like action. We demonstrated that quetiapine and norquetiapine interact *in vitro* and *in vivo* with several targets that may mediate the overall clinical effects of quetiapine. Quetiapine and norquetiapine both exhibit antidepressant-like and anxiolytic-like properties in preclinical models, and norquetiapine contributes significantly to the overall *in vivo* activity of quetiapine. It seems likely that the pharmacological interactions of the combination of quetiapine and norquetiapine at several targets, including NET, 5-HT_{1A} and 5-HT_2 receptors, mediate the antidepressant and anxiolytic effects observed in patients treated with quetiapine.

Methods

In Vitro Binding Studies

Binding assays were performed using membranes prepared by standard methods from cells stably expressing cloned human targets. Displacement binding was performed using either scintillation proximity assay (SPA) (NET/HEK293F cells and 5-HT_{2C}/CHO-K1 cells) or filtration (5-HT transporter [SERT]/HEK293 cells, dopamine transporter [DAT]/CHO-S cells, D_{2S}/CHO-K1 cells, 5-HT_{1A}/CHO cells and 5-HT_{2A}/CHO cells) using tritiated radioligands (MeNER, mesulergine, MADAM [2-(2-dimethylaminomethyl-phenylsulphanyl)-5-methyl-phenylamine], WIN 35428, raclopride, WAY100635 and MDL100907 respectively). The majority of IC_{50} values were calculated with fitting model 205 in XLfit (IDBS, Guildford, UK). 5-HT_{2A} and 5-HT_{2C} IC_{50} values were calculated using PRISM software by GraphPad (La Jolla, CA, USA). Mean apparent inhibition constant (K_i) values were calculated using the

Cheng–Prusoff equation from data derived from at least three independent experiments. *In vitro* assessment of affinity at glutamate receptors was performed on preparations of rat cerebral cortex tissue. Binding at NMDA receptors was evaluated with [^{3}H]-CGP39653 [^{3}H]-TCP and [^{3}H]-MDL 105,519 binding at kainite receptors was evaluated with [^{3}H]-kainic acid and binding at AMPA receptors was evaluated with [^{3}H]-AMPA according to standard validated protocols under conditions defined by the contractor (Cerep, Poitiers, France; www.cerep.fr). Compounds were evaluated in singlicate across eight concentrations (0.01, 0.1, 0.3, 1, 3, 10, 30 and 100 μM).

In Vitro Functional Studies

Uptake inhibition assays were performed using HEK293F cells stably expressing human NET, SERT and DAT. Cryopreserved cells were re-suspended at 60K per well, centrifuged at 110 g for 1 min and incubated at 37°C for 3 h. Uptake inhibition was measured using the neurotransmitter transporter dye (Molecular Devices Corp., Sunnyvale, CA, USA) by a method slightly modified from that reported by Jorgensen *et al*. 2008. The most significant alteration to the method is that fluorescence intensity was evaluated on an Envision reader (PerkinElmer, Waltham, MA, USA). Data were analysed by calculating the % effect with respect to total (0.5% DMSO final) and background signals. D_{2S} pA_2 was measured by the ability of a compound to inhibit the response to 3 μM dopamine (~EC_{80}), using a GTPγS filtration binding assay similar to the method previously described by Lazareno (1999; Hudzik *et al*., 2008). 5-HT_{1A} agonist activity (potency and maximal concentration [E_{max}]) was determined with a GTPγS SPA binding assay using membranes derived from CHO cells stably expressing recombinant human 5-HT_{1A} receptors. Assay conditions are based on those previously reported (Jerning *et al*., 2002), though modified to an SPA format. An efficacy of 100% was defined as the maximal response to 5-HT. 5-HT_{2A} and 5-HT_{2C} antagonist activity was measured with a FLIPR-based method, as previously reported (Porter *et al*., 1999) using cell lines expressing 5-HT_{2A} (PerkinElmer # ES-313C) and 5-HT_{2C} (PerkinElmer # ES-315-CV) receptors.

In Vivo Methods

Animal husbandry practices. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care, and all testing procedures were performed using protocols approved by the Institutional Animal Care and Use Committee at AstraZeneca R&D Wilmington, in accordance with *The Guide for the Care and Use of Laboratory Animals*. Unless noted otherwise, all animals were maintained in rooms with constant temperature (approximately 22°C) and a 12 h light/dark cycle, with free access to food and water. The authors consulted the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.,* 2010) and the *BJP* editorial explaining how this applies to pharmacological studies (McGrath *et al*., 2010).

Mouse forced swimming test. Male BALB/c mice from Jackson Laboratories (Bar Harbor, ME, USA) were used in this study (10 animals per dose per treatment). Upon receipt, mice were assigned unique identification numbers (tail marked) and were group-housed in OptiMICE cages. All animals remained housed in groups of four during the study. Mice were acclimatized to the colony room for at least 1 week before being tested and were subsequently tested at an average age of 8–9 weeks. During the period of acclimatization, mice were examined on a regular basis, handled and weighed to assure adequate health and suitability. In each test, animals were randomly assigned across treatment groups.

The forced swimming test (FST) consisted of one 6 min session of forced swimming in individual opaque cylinders (15 cm tall × 10 cm wide, 1000 mL beakers) containing fresh tap water at a temperature of 23°C ± 2°C and depth of 12 cm (approximately 800 mL) for each test animal. The time the animal spent immobile was recorded over the 6 min trial. Every 1 min, the cumulative immobility time was recorded from the start of the session and noted on the data record sheet. Immobility was defined as the postural position of floating in the water. The animals were generally observed with the back slightly hunched and the head above water with no movements or small, stabilizing movements of the limbs. After the FST, each animal was placed in a pre-heated cage with a heating pad and allowed to dry. All mice were killed on completion of the studies.

Sertraline (20 mg·kg^{-1}, dissolved in sterile injectable water) was administered i.p. 30 min before testing. All other test substances were dissolved in saline and administered s.c. 60 min before testing at a dose volume of 10 mL·kg^{-1}. Each test substance was evaluated in conjunction with a separate sertraline and saline control. Data were analysed by ANOVA followed by Fisher's protected least significant difference *post hoc* comparisons. An effect was considered significant if $P < 0.05$. Statistical outliers that fell above or below 2 SDs from the mean were removed from the final analysis.

Rat learned helplessness test. Rat learned helplessness (LH) was performed as previously reported (Hudzik *et al*., 2011). Male Wistar rats (Charles River, Wilmington, MA, USA) weighed approximately 250–300 g at the time of testing (12 per treatment group). All data on activity in standard shuttle cages (Med Associates, St. Albans, VT, USA), fitted with a grid floor and partitioned chambers, were monitored and stored. Electric shock (1.5–2 mA) was delivered to the floor of the cage by digitally controlled shock output devices interfaced to the monitoring microprocessor. A single episode of induction was produced in each subject by partitioning them to one side of the shuttle cage and randomly delivering electrical stimulation (10 s duration) to the floor of the cage every 2, 5 or 10 s (until 90 shocks were delivered). Each drug was administered (i.p., 1 mL·kg^{-1} in 0.9% NaCl) immediately after induction, again that same evening and on the following day.

Avoidance training trials were conducted in open partition shuttle cages 48 h after induction. A conditioning stimulus (5 s tone accompanied by lamp illumination on the occupied side of the cage) was presented 3 s before an electrical stimulation of the cage floor. Entry into the opposite side of the shuttle cage before shock delivery resulted in the end of the trial (avoidance). If a shock was delivered, entry into

the opposite side of the cage resulted in termination of the shock and the conditioned stimulus (escape). A 30 s inter-trial interval was employed. Remaining on the side of the cage to which the shock was delivered for the full 3 s period constituted escape failure. Avoidance training trials were conducted on two consecutive days (3 and 4), lasted 40 min each and consisted of a maximum of 55 trials.

For each treatment there were two (drug or vehicle i.p.) injections per day, for 3 days, and then one injection on the fourth day. Data from trials on days 3 and 4 were combined and analysed by global ANOVA followed by Dunnett's *post hoc* comparisons. An effect was considered significant if $P < 0.05$.

Rat punished responding. Rat punished responding was performed as previously reported (Hudzik *et al.*, 2011). Male Long–Evans rats weighing 375–425 g were maintained at 80–90% of their free-feeding weights by limiting the feeding after the experimental session. For any given drug test, rats whose responses were most stable were chosen from a larger pool of animals trained as previously described. Pilot doses were tested beforehand in different subjects, and at least eight animals were used for each data point.

Briefly, standard two-lever operant chambers (Med Associates) were fitted with two retractable response levers and a stimulus lamp over each of the levers. A pellet dispenser delivered 45 mg of food pellets (Bio-Serv, Frenchtown, NJ, USA) to a cup located inside the chamber, below and between the two response levers. A lamp at the top and back of the chamber served as the house light. The grid floors of the operant chambers were interfaced to shock generators and scramblers (Med Associates). The unsuppressed response components (unpunished) lasted 2 min, and the suppressed response components (punished) lasted 3 min. In unpunished components, houselights and stimulus lamps were on, the lever on the left-hand side was extended and a food pellet was delivered after an average of 17 responses on the lever in the chamber (VR17 schedule; range, 3–40 responses). The punished components followed unpunished components. During the punished component, only the right-hand lever was extended into the chamber, and the stimulus lamps and houselights were turned on and off at 1 s intervals, in succession. In the punished component, food was also available under a VR17 schedule, but electrical current (0.5 s duration) was delivered to the grid floor of the chamber under an independent VR17 schedule. The level of the current was adjusted for each subject until response in the suppressed component was reduced to 5–10% that of the unpunished component (range, 0.2–0.75 mA) in order to prevent a 'floor effect'. Unpunished and punished components were separated by 10 s timeout periods. The 2 min unpunished and 3 min punished components alternated until five of each were completed. The rate of responding (responses s^{-1}) in unpunished and punished components and the number of shocks delivered were recorded. Data were analysed by omnibus ANOVA followed by Dunnett's *post hoc* comparisons. An effect was considered significant if $P < 0.05$.

Once animals were trained to a stable baseline for three consecutive days, drug testing began. Norquetiapine (0.3, 1, 2, 5 and 10 $mg \cdot kg^{-1}$, $n \geq 6$ per dose) was dissolved in saline and delivered s.c. at 1 $mL \cdot kg^{-1}$, 15 min before testing. Quetiapine (2.5, 5, 10 and 20 $mg \cdot kg^{-1}$, $n \geq 8$ per dose) was formulated in distilled water plus lactic acid drops (pH > 2.5) to dissolution and delivered p.o. at 2 $mL \cdot kg^{-1}$, 60 min before testing. Diazepam in an Abbott's cocktail (10% ethanol, 40% propylene glycol and 50% water) stock solution of 5 $mg \cdot mL^{-1}$ was diluted to dosing volume (0.3, 1 and 3 $mg \cdot kg^{-1}$, $n \geq 3$ per dose) with a 50% concentration of Abbott's cocktail and delivered 30 min before testing. In combination studies, WAY100635 was dissolved in saline and delivered at 0.1 $mg \cdot kg^{-1}$, s.c., alongside the test drug. Responses were normalized to the vehicle control for each experiment. Data from combination studies were analysed by global ANOVA followed by *post hoc t*-test comparisons. An effect was considered significant if $P < 0.05$.

Elevated plus maze with rats from prenatally stressed dams. The procedure used to evaluate elevated plus maze performance of rats from prenatally stressed dams is described in detail by Peters *et al.* (2011). In short, male Sprague–Dawley rats born in-house to prenatally stressed dams (Charles River) were housed singly in an animal room with constant temperature and a 24 h light/dark cycle, on restricted food but with free access to water. On the test day, rats were placed in the centre of the maze facing an open arm, and behaviour was recorded for exactly 5 min. The % time spent in the open arms, the % entries into the open and closed arms and the total number of entries into the open and closed arms were recorded. The rats were dosed s.c. with either vehicle (saline), quetiapine or norquetiapine (5 or 10 $mg \cdot kg^{-1}$ in saline and lactic acid to dissolve them, pH adjusted with sodium bicarbonate to pH > 5) 15 min before testing in the elevated plus maze. The effects of drug treatment in the elevated plus maze were assessed using a one-way ANOVA followed by Dunnett's multiple comparison. The effect of stress in the vehicle-treated animals was assessed with a one-tailed *t*-test.

In vivo receptor occupancy. Male–Long Evans rats (Charles River), weighing approximately 250–300 g at the time of study, were used. After acclimatization to the animal vivarium, rats were anaesthetized with isoflurane, and a 2–3 French catheter was inserted into the jugular vein 1–3 days before the i.v. injection of the radioligand. The tritiated NET ligand, (*S*,*S*)-[^{3}H]-MeNER, was prepared by an adaptation of the previously described method for synthesis of the corresponding PET ligand, (*S*,*S*)-[^{11}C]-MeNER (Schou *et al.*, 2003), to a radiochemical purity of 99% and specific activity of 81 $Ci \cdot mmol^{-1}$.

Before the experiment, animals were weighed and transferred to pans without bedding, food or water. Animals (n = 6 per treatment group) were then administered the drug at the prescribed doses (s.c.). Thirty minutes later, animals were dosed i.v. with 7.5 μCi of [^{3}H]-MeNER in 1 mL of saline. Ninety minutes after administration of [^{3}H]-MeNER, animals were killed by decapitation, brains were removed and the locus coeruleus and striatum were dissected and frozen on dry ice. The tissue was weighed and solubilized overnight in 2 mL of Soluene (PerkinElmer, Waltham, MA, USA). The following day, 5 mL of Ultima Gold Scintillation Cocktail

(PerkinElmer) was added, and the samples were counted in a Packard Tri-Carb Scintillation Counter (PerkinElmer). Raw data were converted to reductions in radioactivity (fmol·mg^{-1} tissue), and relative occupancy was reported. Relative occupancy is defined as the amount of specific binding (fmol·kg^{-1} locus coeruleus – fmol·kg^{-1} striatum) displaced versus vehicle-treated animals (Wilson *et al.*, 2003).

Drugs

Quetiapine, norquetiapine (11-(piperazin-1-yl)dibenzo[*b*,*f*][1,4]thiazepine), [^{3}H]-MDL100907 and [^{3}H]-MADAM [2-(2-dimethylaminomethyl-phenylsulphanyl)-5-methyl-phenyl-amine] were synthesized at AstraZeneca Pharmaceuticals LP. Clozapine, risperidone, raclopride tartrate, GBR 12909 (1-(2-[bis(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl) piperazine) dihydrochloride, WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide) maleate, (*R*)-(+)-8-hydroxy-DPAT hydrobromide (8-OH-DPAT), imipramine hydrochloride, desipramine hydrochloride, mianserin hydrochloride and nisoxetine hydrochloride were purchased from Sigma Aldrich Corp (St. Louis, MO, USA). Reboxetine mesylate, duloxetine hydrochloride, olanzapine and ziprasidone were purchased from AK Scientific (Mountain View, CA, USA). [*N*-Methyl^{3}H]-WIN 35428 was purchased from PerkinElmer (Waltham, MA, USA). [^{3}H]-mesulergine was purchased from GE/Amersham (Fairfield, CT, USA). Aripiprazole was isolated from tablets in the Chemistry Department at AstraZeneca.

Results

In Vitro Binding and Function

Dopamine D_2 receptors. Norquetiapine and quetiapine exhibited lower affinity and lower functional potency at D_2 receptors than other antipsychotics (e.g. norquetiapine and quetiapine pK_i = 7.25 ± 0.25 and 7.23 ± 0.40, respectively, vs. risperidone pK_i = 9.79 ± 0.20).

Monoamine transporters (NET, SERT and DAT). Norquetiapine inhibited NET binding with high affinity (pK_i = 7.54 ± 0.05) and was a potent inhibitor in functional uptake assays (pK_i = 7.47 ± 0.17); quetiapine had no measurable NET binding or functional blocking properties in these tests (Table 1). In contrast to norquetiapine, the reference antipsychotics generally did not have potent NET binding or uptake-blocking potency. Olanzapine and risperidone had no detectable binding at NET, and clozapine (pK_i = 5.44 ± 0.04) and aripiprazole (5.93 ± 0.02) were 30–100 times less potent than norquetiapine. Ziprasidone had moderate NET binding affinity (pK_i = 6.46 ± 0.05) and uptake inhibition (pK_i = 7.17 ± 0.34). Norquetiapine affinity at NET more closely resembled the affinity of several reference antidepressants (e.g. duloxetine and imipramine, Table 1).

At the other monoamine transporters (SERT and DAT), both quetiapine and norquetiapine had low binding affinity and very weak uptake inhibition (Table 1). Similarly, the other antipsychotics (e.g. olanzapine, clozapine and risperidone) generally had no measurable SERT or DAT binding affinity and low potency (pK_i < 7) uptake inhibition.

Table 1

Drug activity in *in vitro* binding and functional uptake assays of cloned human (h) monoamine transporters.

Drug	hNET binding pK_i ± SD	hNET uptake pK_i ± SD	hSERT binding pK_i ± SD	hSERT uptake pK_i ± SD	hDAT binding pK_i ± SD	hDAT uptake pK_i ± SD
Quetiapine	IA	IA	IA	IA	IA	IA
Norquetiapine	7.54 ± 0.05	7.47 ± 0.17	IA	5.94 ± 0.19	IA	IA
Antipsychotics						
Olanzapine	IA	5.59 ± 0.20	IA	5.79 ± 0.10	IA	IA
Clozapine	5.44 ± 0.04	6.38 ± 0.09	IA	6.29 ± 0.37	IA	IA
Risperidone	IA	IA	IA	5.54 ± 0.07	IA	IA
Ziprasidone	6.46 ± 0.05	7.17 ± 0.34	IA	6.67 ± 0.09	6.69 ± 0.08	6.92 ± 0.06
Aripiprazole	5.93 ± 0.02	6.05 ± 0.15	5.85 ± 0.30	6.85 ± 0.29	IA	6.60 ± 0.26
Antidepressants						
Duloxetine	7.62 ± 0.04	7.65 ± 0.19	10.64 ± 0.14	9.23 ± 0.20	6.05 ± 0.07	6.96 ± 0.25
Desipramine	8.93 ± 0.34	8.89 ± 0.02	7.38 ± 0.28	7.72 ± 0.06	IA	5.34 ± 0.12
Imipramine	7.24 ± 0.03	7.84 ± 0.04	9.44 ± 0.17	8.94 ± 0.25	IA	5.15 ± 0.08
Mianserin	6.81 ± 0.02	7.60 ± 0.18	IA	6.03 ± 0.12	4.96 ± 0.11	IA
Reference agents						
Atomoxetine	8.35 ± 0.05	8.83 ± 0.21				
Reboxetine	8.73 ± 0.29	8.83 ± 0.14				
Nisoxetine	8.74 ± 0.43	8.89 ± 0.44				
Escitalopram			9.29 ± 0.18	9.20 ± 0.28		
GBR 12909					8.81 ± 0.27	8.06 ± 0.25

IA, inactive; hNET, noradrenaline transporter; hSERT, 5-HT transporter; hDAT, dopamine transporter.
All experiments were carried out with n = 3 or more and are presented as mean pK_i ± SD.

Not surprisingly, most established antidepressants had high SERT affinity. High-affinity SERT binding was observed for duloxetine (pK_i = 10.64 ± 0.14) and imipramine (9.44 ± 0.17) and moderate-affinity SERT binding was observed for desipramine (7.38 ± 0.28). These antidepressants also displayed high-to-moderate potency functional SERT inhibition (Table 1). The SERT uptake pK_i values for duloxetine, imipramine and desipramine were 9.23 ± 0.20, 8.94 ± 0.25, and 7.72 ± 0.06 respectively. All compounds evaluated exhibited low potency at DAT.

Selected 5-HT receptors (5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$). 5-HT$_{1A}$ receptors: both quetiapine and norquetiapine bound to 5-HT$_{1A}$ receptors with moderate affinity, and both were low-potency, full agonists (Table 2). An agonist potency for norquetiapine (pEC_{50}) of 5.47 with an E_{max} of 90% was observed. Quetiapine exhibited an agonist pEC_{50} value of 4.77 and an E_{max} of 89%. Although higher affinity and potency values were observed with other antipsychotics, lower corresponding maximal efficacy values were observed (e.g. a pEC_{50} of 7.87 ± 0.28 and E_{max} of 65% were observed for aripiprazole).

5-HT$_{2A}$ receptors: norquetiapine and quetiapine had moderate to high affinity for the human 5-HT$_{2A}$ receptor (Table 2). Norquetiapine had a pK_i value of 8.29 ± 0.47, while quetiapine had a pK_i value of 7.54 ± 0.30. Other antipsychotics generally had higher affinity for 5-HT$_{2A}$ receptors (e.g. pK_i for olanzapine = 9.29 ± 0.35, clozapine = 8.61 ± 0.52 and ziprasidone = 9.49 ± 0.39). Established antidepressants generally had low affinity for 5-HT$_{2A}$ receptors. Duloxetine, desipramine and imipramine all had pK_i values between 6 and 6.5. In contrast, mianserin had moderate to high affinity for 5-HT$_{2A}$ receptors (pK_i = 8.89 ± 0.29). In functional assays, norquetiapine and quetiapine were antagonists of moderate potency at 5-HT$_{2A}$ receptors (quetiapine pIC_{50} = 6.18 ± 0.24, norquetiapine = 7.23 ± 0.18, vs. mianserin = 7.45 ± 0.26).

5-HT$_{2C}$ receptors: Quetiapine had low affinity (pK_i = 5.55 ± 0.26) at 5-HT$_{2C}$ receptors, whereas norquetiapine was more potent (pK_i = 7.12 ± 0.12) (Table 2). All other antipsychotics had higher affinity at 5-HT$_{2C}$ receptors (Table 2). Ziprasidone notably had high affinity (pK_i = 9.48 ± 0.23) at 5-HT$_{2C}$ receptors, 100-fold greater than norquetiapine. Established antidepressant drugs had low affinity (pK_i = 6.5 or lower) at 5-HT$_{2C}$ receptors (Table 2). However, mianserin had moderate to high affinity (pK_i = 8.87 ± 0.09) at 5-HT$_{2C}$ receptors (Table 2). In functional assays, norquetiapine and quetiapine were low-potency antagonists at 5-HT$_{2C}$ receptors (quetiapine pIC_{50} = 4.44 ± 0.87, norquetiapine = 6.90 ± 0.20, vs. mianserin = 7.12 ± 0.15).

Excitatory amino acid receptors (AMPA, kainate or NMDA). Quetiapine and norquetiapine were also evaluated for affinity at excitatory amino acid receptors. Neither displayed affinity below 50 μM at known AMPA, kainate or NMDA receptor binding sites (Table S1).

In Vivo NET Receptor Occupancy

[^{3}H]-MeNER-specific binding was measured in the locus coeruleus, a brain region reported to exhibit a high density of binding sites in rodents (Ghose *et al.*, 2005). An excess of the selective NET ligand, nisoxetine (30 mg·kg^{-1}, s.c.), displaced 80% of total [^{3}H]-MeNER binding in the locus

Table 2

Drug activity in *in vitro* binding and functional data for cloned human (h) D_2 and 5-HT receptors

Drug	hD$_2$ binding pK_i ± SD	hD$_2$ antagonist GTPγS pIC_{50} ± SD	h5-HT$_{1A}$ binding pK_i ± SD	h5-HT$_{1A}$ agonist GTPγS pEC_{50} ± SD	h5-HT$_{1A}$ agonist GTPγS % E_{max} ± SD	h5-HT$_{2A}$ binding pK_i ± SD	h5-HT$_{2C}$ binding pK_i ± SD
Quetiapine	7.25 ± 0.25	6.33 ± 0.24	5.74 ± 0.10	4.77 ± 0.22	89 ± 8	7.54 ± 0.30	5.55 ± 0.26
Norquetiapine	7.23 ± 0.40	6.10 ± 0.25	6.24 ± 0.08	5.47 ± 0.16	90 ± 8	8.29 ± 0.47	7.12 ± 0.12
Antipsychotics							
Olanzapine	8.87 ± 0.38	7.15 ± 0.36	IA	IA	IA	9.29 ± 0.35	7.93 ± 0.17
Clozapine	7.71 ± 0.07	6.57 ± 0.28	6.27 ± 0.01	6.00 ± 0.23	48 ± 5	8.61 ± 0.52	7.88 ± 0.25
Risperidone	9.79 ± 0.20	7.87 ± 0.26	6.83 ± 0.07	IA	IA	8.83 ± 0.50	8.05 ± 0.17
Ziprasidone	9.80 ± 0.13	7.85 ± 0.39	8.21 ± 0.07	7.67 ± 0.23	73 ± 2	9.49 ± 0.39	9.48 ± 0.23
Aripiprazole	10.49 ± 0.36	8.09 ± 0.27	7.94 ± 0.14	7.87 ± 0.28	65 ± 3	9.13 ± 0.69	7.48 ± 0.12
Antidepressants							
Duloxetine	6.60 ± 0.38	4.77 ± 0.19	IA	IA	IA	6.00 ± 0.06	5.53 ± 0.02
Desipramine	7.00 ± 0.48	5.44 ± 0.35	6.14 ± 0.21	IA	IA	6.32 ± 0.12	6.20 ± 0.02
Imipramine	6.86 ± 0.18	5.40 ± 0.08	6.12 ± 0.29	IA	IA	6.46 ± 0.13	6.48 ± 0.03
Mianserin	7.46 ± 0.09	4.97 ± 0.40	5.63 ± 0.11	5.06 ± 0.04	98 ± 1	8.89 ± 0.29	8.87 ± 0.09
Reference agents							
Raclopride	9.84 ± 0.43	8.48 ± 0.24					
Buspirone			7.19 ± 0.22	7.27 ± 0.11	70 ± 3		
WAY100635			9.60 ± 0.49	IA	IA		
8-OH-DPAT			7.93 ± 0.08	8.83 ± 0.22	94 ± 1		

IA, inactive.

All experiments were carried out with n = 3 or more determinations and are presented as the mean pK_i or pIC_{50} or E_{max} ± SD.

coeruleus (data not shown). Dose-dependent displacement of specific [^{3}H]-MeNER binding by the NET inhibitors, reboxetine, desipramine and norquetiapine, was determined (Figure 1). The relative occupancy of reboxetine, desipramine and norquetiapine yielded ED_{50}s of approximately 0.05, 0.15 and 2 mg·kg^{-1}, respectively.

Mouse forced swimming test. Norquetiapine significantly reduced immobility in BALB/c mice at 30 mg·kg^{-1} (Figure 2), whereas quetiapine was inactive (data not shown). Desipramine also significantly reduced immobility at 30 mg·kg^{-1}, s.c., whereas reboxetine significantly reduced immobility at both 10 and 30 mg·kg^{-1}. The positive control antidepressant, sertraline, was active in all tests conducted.

Rat learned helplessness test. Vehicle control animals exhibited significantly more escape failures than non-inducted (no shock) controls in all test cohorts (Figure 3). Treatment with the positive control, imipramine, resulted in significantly fewer escape failures than the vehicle-only group in all cohorts. The low doses of all test compounds (norquetiapine, desipramine and reboxetine) as well as the high dose of reboxetine (30 mg·kg^{-1}) were inactive in the LH test paradigm. In contrast, animals treated with the high dose of norquetiapine (5 mg·kg^{-1}) and the high dose of desipramine (10 mg·kg^{-1}) demonstrated statistically significant reductions in escape failures.

Rat punished responding. Both quetiapine and norquetiapine were active in the rat punished responding test, increasing the rate of punished responding to a similar extent as the reference anxiolytic, diazepam (Figure 4). The doses of quetiapine (10 mg·kg^{-1}) and norquetiapine (5 mg·kg^{-1}) exhibiting the most efficacy in potentiating the response rate within the punished state were used in a follow-up study.

The effect of norquetiapine (5 mg·kg^{-1}) on punished responding was blocked by co-administration with the 5-HT$_{1A}$ antagonist, WAY100635. Although a similar trend was observed with the quetiapine (10 mg·kg^{-1}) and WAY100635 combination treatment, a comparison did not reach statistical significance. In a separate experiment, the 5-HT$_{1A}$ agonist, (*R*)-(+)-8-OH-DPAT (0.03 mg·kg^{-1}), was also found to be active in punished responding, and again, this effect was blocked by combination with 0.1 mg·kg^{-1} WAY100635 (data not shown).

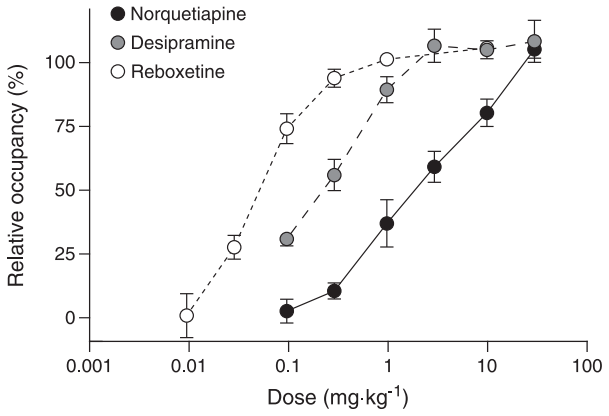

Figure 1

Occupancy of rat noradrenline transporter (NET) in locus coeruleus following s.c. administration of norquetiapine, desipramine or reboxetine. Data are presented as mean relative occupancy ± SEM (n = 6 animals per treatment group).

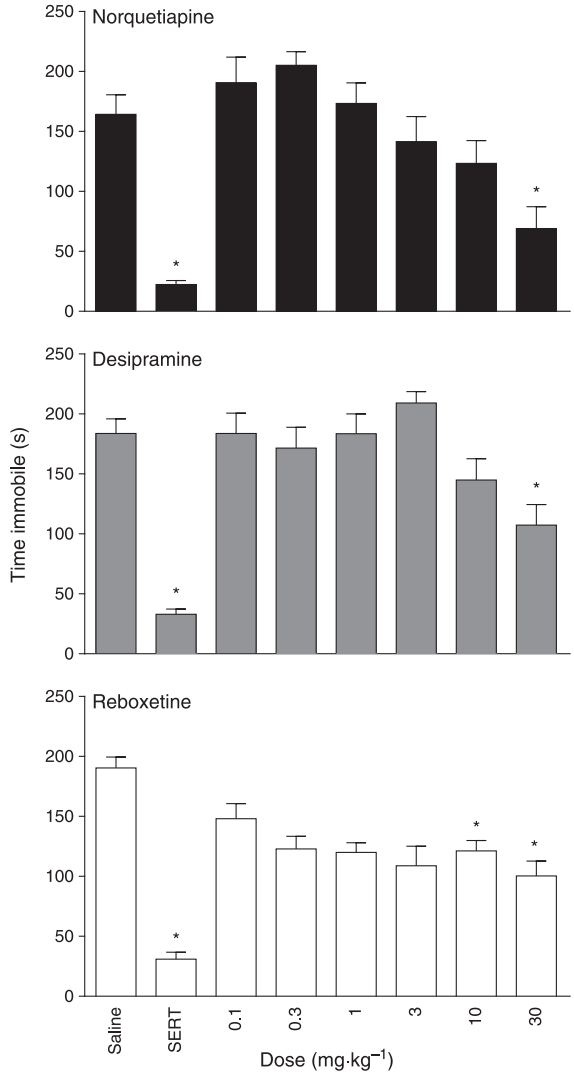

Figure 2

Effects of norquetiapine, desipramine or reboxetine in the forced swim test in male BALB/c mice. Vehicle (saline) and sertraline were used as controls in each experiment. Data are presented as mean immobility time ± SEM (n = 10 animals per treatment group). Note: * indicate a mean value significantly different from the vehicle ($P < 0.05$).

Rat elevated plus maze

Norquetiapine significantly reversed the suppressed spontaneous exploratory behaviour into open arms observed in rats derived from dams of prenatally stressed mothers. A reversal of the behavioural deficit of % time spent in open arms was observed at both doses (5 and 10 mg·kg^{-1}). No reversal of the deficit was observed with quetiapine at either dose (5 and 10 mg·kg^{-1}) (Figure 5).

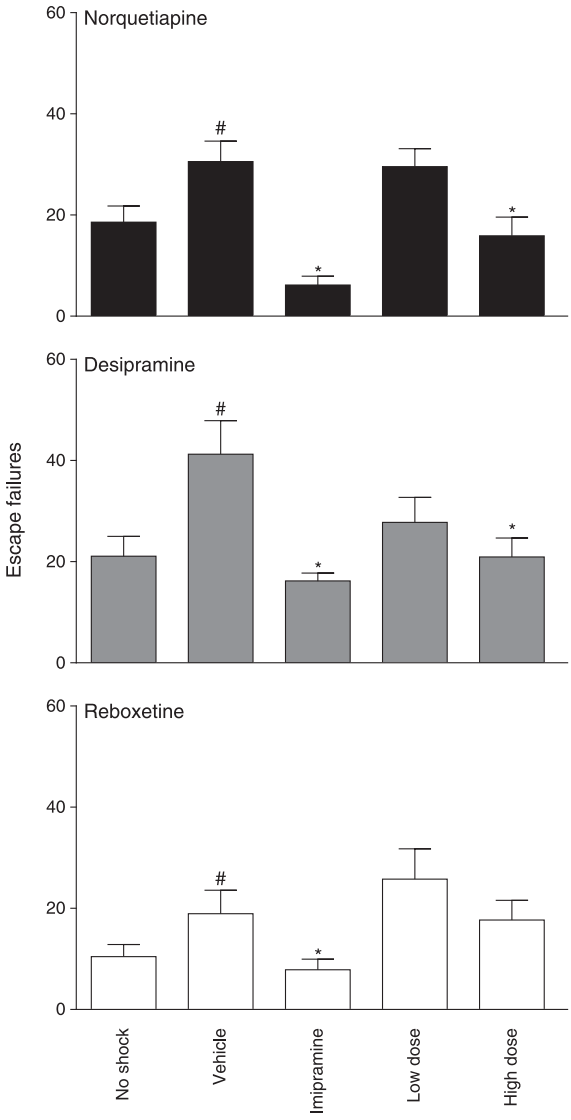

Figure 3

Effects of norquetiapine, desipramine or reboxetine on learned helplessness in male Wistar rats. Non-inducted animals (no shock), saline only (vehicle) and imipramine-treated controls were included in each experiment. A low dose (norquetiapine, 0.5 mg·kg^{-1}; desipramine, 3 mg·kg^{-1}; and reboxetine, 10 mg·kg^{-1}) and a high dose (norquetiapine, 5 mg·kg^{-1}; desipramine, 10 mg·kg^{-1}; and reboxetine, 30 mg·kg^{-1}) of each test compound were evaluated. Data are presented as mean escape failures ± SEM (n = 12 animals per treatment group). Note: * indicate a mean value significantly different from the vehicle ($P < 0.05$); # indicate an uninduced (no shock) mean value significantly ($P < 0.05$) different from shock + vehicle (vehicle).

Discussion

The current study demonstrates that quetiapine and its major active human metabolite, norquetiapine, interact with molecular targets of established antidepressants and anxiolytics (e.g. NET, 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_{1A}$ receptors), which translates to antidepressant- and anxiolytic-like activity in behavioural models. We focus here on the established monoamine targets of antidepressant drugs as quetiapine and norquetiapine do not interact with ligand binding sites of excitatory amino acid receptors associated with novel potential antidepressant drugs such as ketamine. These results extend previous findings on the *in vitro* and *in vivo* pharmacology of norquetiapine (Goldstein *et al.*, 2007; Hudzik *et al.*, 2008; Jensen *et al.*, 2008) and quetiapine (Saller and Salama, 1993) and provide a mechanistic basis for the clinical efficacy of quetiapine in mood and anxiety disorders (McIntyre *et al.*, 2005; Hirschfeld *et al.*, 2006; Weisler *et al.*, 2009).

Norquetiapine has been identified as a major active metabolite of quetiapine in humans; however, the metabolism of quetiapine in rodents varies depending on route of administration, species and strain (Winter *et al.*, 2008). The formation of norquetiapine from quetiapine is considerably lower in rats and mice compared with humans, and in the present study, production of norquetiapine from quetiapine was minimized by avoiding p.o. administration.

The observations of potent NET binding and functional inhibition with norquetiapine, and low-affinity binding and weak functional inhibition with quetiapine, confirm previous reports (Goldstein *et al.*, 2007; Jensen *et al.*, 2008). Given that NET inhibition is an established mechanism associated with antidepressant drug action, this is consistent with the notion that norquetiapine could contribute significantly to the antidepressant effect of quetiapine through inhibition of NET. The low affinity and negligible functional inhibition at SERT and DAT by both norquetiapine and quetiapine suggest that interaction at these targets does not play a significant role in the composite pharmacology of quetiapine/norquetiapine. In animal models, the demonstration of antidepressant-like activity similar to desipramine, coupled with occupancy of NET at behaviourally relevant doses, supports the hypothesis that NET inhibition by norquetiapine contributes significantly to antidepressant-like effects seen in preclinical studies. Given that quetiapine administration to rodents by the i.p. or s.c. route produces only minimal norquetiapine production and that quetiapine is inactive in FST and LH, it is likely that norquetiapine is both necessary and sufficient to produce the antidepressant-like effect.

It should be noted that norquetiapine is more potent in *in vivo* models of antidepressant drug action than might be predicted from NET inhibition alone. Direct interactions with adrenergic receptors are unlikely to account for this activity as norquetiapine is less potent at adrenergic receptors than quetiapine (Jensen *et al.*, 2008). Norquetiapine is considerably less potent than desipramine or reboxetine at inhibiting NET occupancy *in vivo*, whereas all three compounds were roughly equipotent in the mouse FST. Both norquetiapine and desipramine were of similar potency in the LH. It is possible that other pharmacological properties of norquetiapine may contribute in combination with NET inhibition to produce the unexpected potency in the FST and LH. The demonstrated interactions of quetiapine and norquetiapine with 5-HT$_2$ receptors may be of particular relevance in this regard. Quetiapine and norquetiapine were antagonists of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, and it has been shown previously that antagonists of both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors can potentiate antidepressant-like

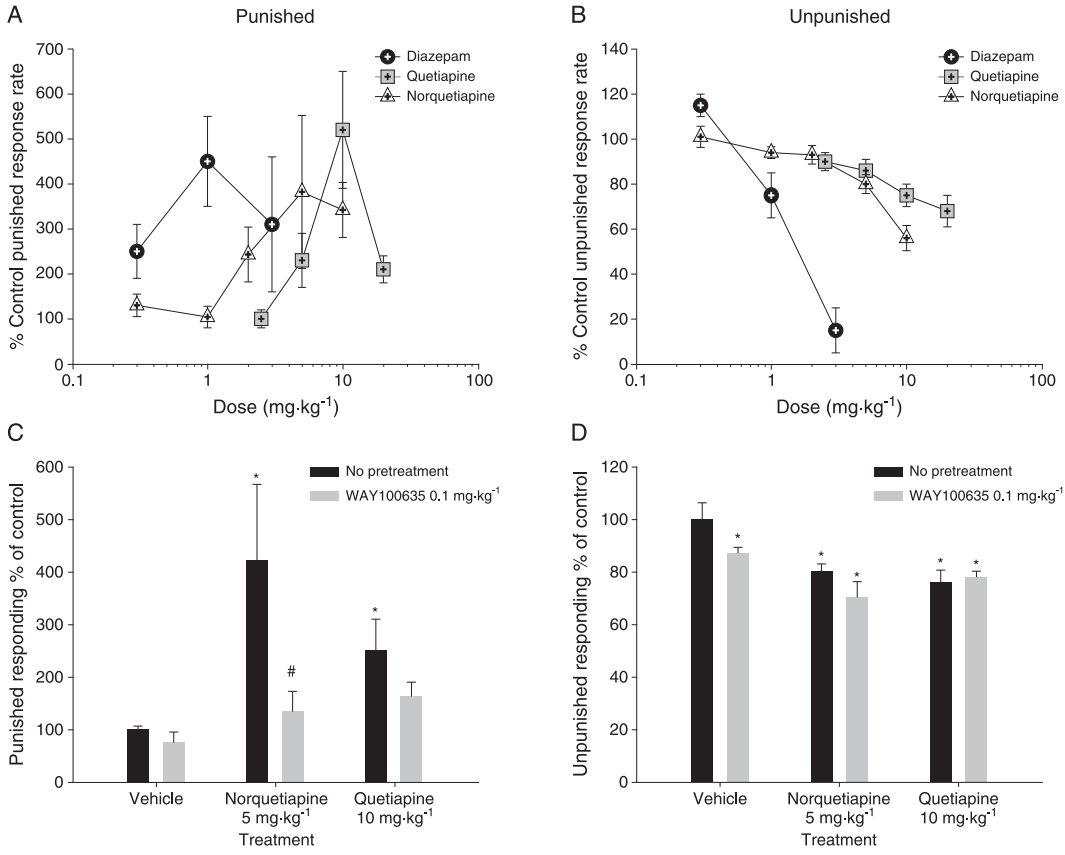

Figure 4

Effects of norquetiapine ($n \geq 6$ per dose), quetiapine ($n \geq 8$ per dose) and diazepam ($n \geq 3$ per dose) on punished responding in male Long–Evans rats. Punished (A) and unpunished (B) response rates across doses are displayed as means ± SD. In combination studies (C and D), peak efficacy doses of norquetiapine (5 mg·kg^{-1}) and quetiapine (10 mg·kg^{-1}) were delivered either without or following WAY100635 (0.1 mg·kg^{-1}) co-administration. Note:* indicate a mean value significantly different from the vehicle ($P < 0.05$); # indicate WAY100635 pretreatment mean value significantly ($P < 0.05$) different from test drug only.

activity in the FST (reviewed by Carr and Lucki 2011). Moreover, antagonists of both 5-HT$_{2A}$ (Marek *et al.*, 2005) and 5-HT$_{2C}$ receptors (Dekeyne *et al.*, 2008) have been reported to exert both antidepressant and anxiolytic properties in preclinical tests. Norquetiapine was considerably more potent than quetiapine as a 5-HT$_{2C}$ antagonist. If this feature is relevant to the antidepressant effect, then norquetiapine is likely to contribute substantially to clinical efficacy through 5-HT$_{2C}$ antagonism as well as through NET inhibition.

Both quetiapine and norquetiapine exhibited low-potency, full-agonist activity at 5-HT$_{1A}$ receptors. In contrast, the other antipsychotics tested generally displayed higher potency, but lower efficacy (i.e. partial agonism). It is unclear how low-potency, full-agonist activity at 5-HT$_{1A}$ receptors in isolation might translate into clinical activity. There are a few examples of 5-HT$_{1A}$ agonists in the azapirone class that have been used in clinical settings, and their utility as monotherapies for mood disorders remains unconfirmed (Chessick *et al.*, 2006). Rather, the utility of azapirone class agents like buspirone may be more appropriate as augmentation therapies (Gaynes *et al.*, 2012). Thus, the contribution of this mechanism to the composite pharmacology of quetiapine/norquetiapine may be an essential feature critical to the broad effectiveness of quetiapine in the treatment of mood disorders.

Punished responding is a well-established model predicting anxiolytic-like effects of GABAergic and serotonergic drugs (Cryan and Sweeney, 2011). Both quetiapine and norquetiapine were found to be active in this model. Preliminary studies indicate that quetiapine and norquetiapine do not interact with GABA$_A$ or GABA$_B$ receptors (data not shown). The anxiolytic-like effect of norquetiapine was confirmed in the elevated plus maze model, in which quetiapine was inactive. Interestingly, neither the NET inhibitor, atomoxetine, nor the D$_2$ antagonist, raclopride, demonstrated any appreciable activity in the punished responding model (data not shown). Although mixed 5-HT$_2$ antagonists have been reported as active in punished responding, the reported activity of selective 5-HT$_{2A}$ or 5-HT$_{2C}$ antagonists has been far less consistent (Kennett *et al.*, 1994; Martin *et al.*, 2002). As previously reported, the 5-HT$_{1A}$ agonist, (*R*)-(+)-8-OH-DPAT, induced only a modest potentiation of punished responding (Commissaris *et al.*, 2000). Collectively, this suggests that individual components of the norquetiapine pharmacological profile (NET inhibition, D$_2$ antagonism, 5-HT$_2$ antagonism and 5-HT$_{1A}$ agonism) are insufficient for robust activity. However, blockade of norquetiapine's activity on

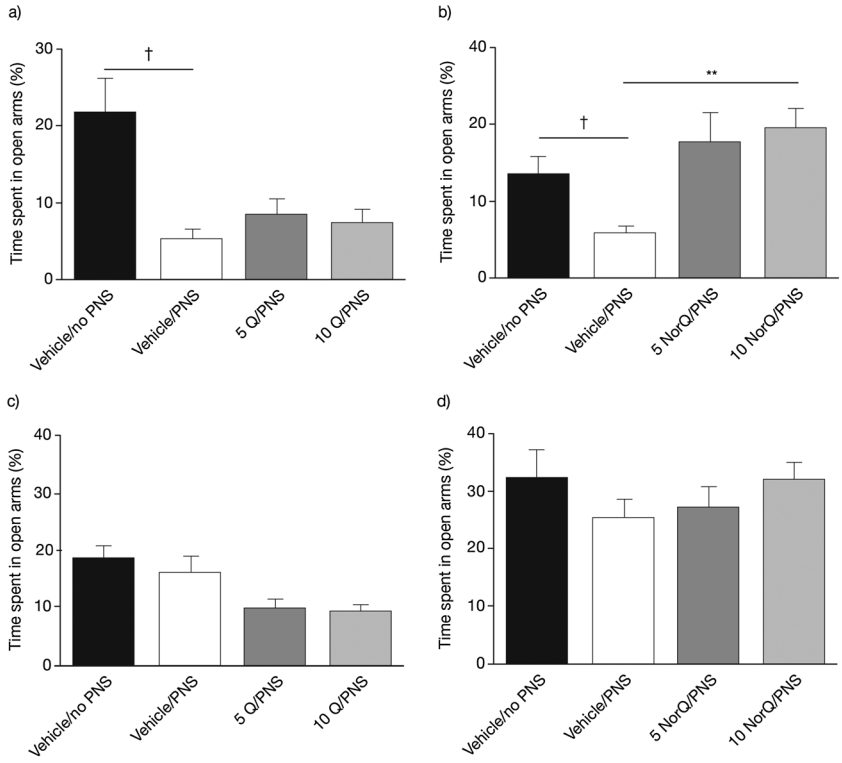

Figure 5

Effects of quetiapine (A and C) and norquetiapine (B and D) following prenatal stress on elevated plus maze performance of adult Sprague–Dawley rats. Prenatal stress suppressed spontaneous exploration of open arms in the elevated plus maze in both experiments; measured as % time spent in open arms († $P \leq 0.01$). (B) Norquetiapine at both 5 and 10 mg·kg^{-1} doses restored the fraction of time spent in open arms (** $P < 0.01$). (D) Neither dose of quetiapine was effective. The data displayed are average ± SEM.

punished responding by concomitant treatment with the 5-HT$_{1A}$ antagonist, WAY100635, suggests that activation of 5-HT$_{1A}$ receptors is necessary for the expression of activity in this model. As already discussed, this may be reflected in the clinical experience attendant with adjunctive use of 5-HT$_{1A}$ agonists.

It is well established that the clinical dosing of antipsychotics reflects their potency at D$_2$ receptors (Seeman *et al.*, 1975), yielding a consistent *in vivo* D$_2$ receptor occupancy of 70–80% (Farde *et al.*, 1992). Thus, the affinity for other targets should be considered in relation to D$_2$ receptor affinity when attempting to understand the importance of engaging a spectrum of targets (see also Bjorkholm *et al.*, 2013, for discussion in relation to psychosis). Because patients taking quetiapine fumarate tablets are exposed to both quetiapine and norquetiapine (Winter *et al.*, 2008), the composite pharmacological activity of quetiapine and norquetiapine at various targets is likely to mediate its clinical profile. The moderate D$_2$ affinity and low-potency D$_2$ antagonism of quetiapine and norquetiapine suggest that other pharmacological characteristics may contribute prominently to quetiapine's broad clinical efficacy, and particularly to its efficacy in mood disorders. The combination of pharmacological activities observed here is not seen with other atypical antipsychotics, which generally have little or no NET inhibition to complement D$_2$ antagonism. Of particular note is the observation that quetiapine demonstrates clinical antidepressant efficacy at doses lower than those required for treating schizophrenia and bipolar mania. Consistent with the finding of NET occupancy at doses showing antidepressant-like efficacy in rodents, PET studies in human volunteers demonstrate NET occupancy following administration of 300 mg of quetiapine, the clinically effective dose in major depressive disorder (Nyberg *et al.*, 2013).

The unique combination of activities (i.e. NET inhibition, 5-HT$_{2A}$/5-HT$_{2C}$ antagonism and 5-HT$_{1A}$ full efficacy combined with low-potency D$_2$ antagonism) is not observed with the other antipsychotic drugs tested in our study. It is noteworthy that several atypical antipsychotics such as clozapine and olanzapine share an analogous core structure to quetiapine and are metabolized at least partially via *N*-dealkylation. Although several distinct pharmacologies have been described for *N*-desmethyl clozapine, neither *N*-desmethyl clozapine nor *N*-desmethyl olanzapine are active NET inhibitors, reinforcing the novelty of the interaction of norquetiapine and the antidepressant/anxiolytic action of quetiapine.

Some antidepressants tested in our studies (e.g. duloxetine and imipramine) had NET binding and functional inhibition similar to norquetiapine. However, no antidepressants had D$_2$ antagonism, 5-HT$_{1A}$ agonism and 5-HT$_{2A/C}$ antagonism similar to the quetiapine/norquetiapine combination. The moderate-binding-affinity/low-potency antagonism at D$_2$ receptors relative to the affinity and potency at 5-HT$_2$ receptors and NET of the quetiapine/norquetiapine combination may be an important attribute for the distinctive clinical activity of quetiapine.

In summary, while the exact mechanism of action of quetiapine in psychiatric disorders is not known, the present studies demonstrate that quetiapine and norquetiapine interact *in vitro* and *in vivo* with multiple known targets (i.e. NET, 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors) of antidepressant drugs. The action of norquetiapine as a NET inhibitor contributes significantly to the overall *in vivo* activity of quetiapine and is distinct from the other antipsychotics examined. Norquetiapine exhibits antidepressant-like properties in preclinical models, and both norquetiapine and quetiapine exhibit anxiolytic-like activity. The activity of quetiapine and norquetiapine at any one of the targets alone may not account for the overall clinical profile of quetiapine in psychosis and mood disorders. Rather, it seems likely that the pharmacological interactions of the combination of quetiapine and norquetiapine at multiple targets mediate the antidepressant effects observed in patients treated with quetiapine.

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Author contributions

A. J. C. designed experiments, interpreted results, and wrote the manuscript. D. W. designed experiments, analysed and interpreted data and wrote the manuscript. C. M. designed and executed experiments (punished responding and elevated plus maze), and analysed and interpreted data. A. Z. coordinated and ran the *in vivo* receptor occupancy studies. T. H. designed and oversaw *in vivo* studies and contributed to the writing. J. L. performed *in vitro* pharmacology studies. S. N. interpreted the results and contributed to the writing of the manuscript. M. W. W. designed experiments and wrote the manuscript.

Conflict of interest

A. J. C. and M. W. W. are employees and shareholders of AstraZeneca. D. W., C. M., A. Z., T. H., J. L. and S. N. were previous employees and shareholders of AstraZeneca.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Table S1 Quetiapine and norquetiapine in glutamate receptor binding assays