BMC Pharmacology

RESEARCH ARTICLE

Open Access

Novel olanzapine analogues presenting a reduced H_1 receptor affinity and retained $5HT_{2A}/D_2$ binding affinity ratio

Somayeh Jafari[1,2], Marc E Bouillon[2], Xu-Feng Huang[1,3], Stephen G Pyne[2] and Francesca Fernandez-Enright[1,3*]

Abstract

Background: Olanzapine is an atypical antipsychotic drug with high clinical efficacy, but which can cause severe weight gain and metabolic disorders in treated patients. Blockade of the histamine 1 (H_1) receptors is believed to play a crucial role in olanzapine induced weight gain, whereas the therapeutic effects of this drug are mainly attributed to its favourable serotoninergic 2A and dopamine 2 ($5HT_{2A}/D_2$) receptor binding affinity ratios.

Results: We have synthesized novel olanzapine analogues **8a** and **8b** together with the already known derivative **8c** and we have examined their respective *in vitro* affinities for the $5HT_{2A}$, D_2, and H_1 receptors.

Conclusions: We suggest that thienobenzodiazepines **8b** and **8c** with lower binding affinity for the H_1 receptors, but similar $5HT_{2A}/D_2$ receptor binding affinity ratios to those of olanzapine. These compounds may offer a better pharmacological profile than olanzapine for treating patients with schizophrenia.

Keywords: Olanzapine, Novel antipsychotics, $5HT_{2A}/D_2$ affinity ratio, H_1 receptors

Background

Antipsychotics are widely prescribed for treating severe psychotic diseases, including schizophrenia, bipolar mania, and delusional disorders [1-3]. Antipsychotic therapy alleviates symptoms of schizophrenia but can produce severe side effects. The side effects of antipsychotic medication vary according to the type of antipsychotic administered. Typical antipsychotics such as haloperidol have a high affinity for dopaminergic subtype 2 (D_2) receptors [4-6]. The blockade of D_2 receptors in the mesolimbic dopamine pathways leads to the reduction of the positive symptoms of schizophrenia such as hallucination and delusion [4,7]. However, inhibition of D_2 receptors in the mesocortical dopamine pathway can cause emotional and cognitive problems [8]. In the nigrostriatal pathways, the reduction of dopamine by typical antipsychotics is associated with an occurrence of extrapyramidal symptoms (EPS), including tardive dyskinesia (TD), dystonia,

akathesia, and Parkinsonism [9]. By blocking D_2 receptors in the mesolimbic dopamine pathway, a reduction of the positive symptoms is observed. Nevertheless, the lack of selectivity of typical antipsychotics in blocking D_2 receptors in the nigrostriatal and the mesocortical pathway leads to the expression of various motor disorders as well as worsening the negative (disturbances in emotion and behaviour) and cognitive symptoms, respectively [10-12]. Atypical antipsychotics (second generation), on the other hand, are not only capable of controlling the positive symptoms of schizophrenia but they are also believed to improve the therapeutic effects on the negative symptoms and cognitive dysfunction [9]. Clinical trials have reported fewer incidents of motor dysfunction with atypical antipsychotic treatment compared to typical antipsychotic medication [13]. Additionally, atypical antipsychotic agents have both serotonin 2A ($5HT_{2A}$) and D_2 receptors antagonist properties while typical antipsychotics mainly bind to the D_2 receptors [14]. A higher affinity of atypical antipsychotics for the $5HT_{2A}$ receptors compared to D_2 receptors has been hypothesized for their enhanced efficacy and reduced incidences of EPS [4].

Tetracyclic compounds such as clozapine (**1**) and olanzapine (**2**) are one of the most prominent representatives

---

* Correspondence: fernande@uow.edu.au
[1]Center for Translational Neurosciences, Illawarra Health and Medical Research Institute, School of Health Sciences, The University of Wollongong, Wollongong, NSW, Australia
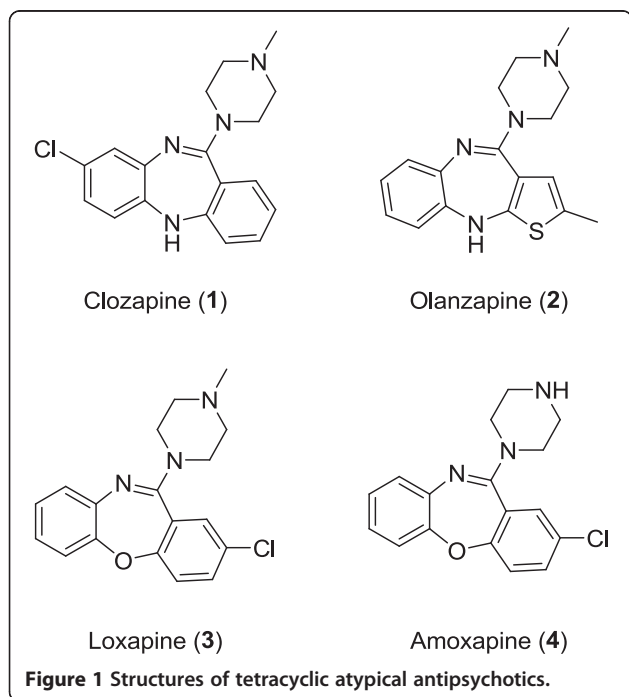[3]Schizophrenia Research Institute, Sydney, NSW, Australia
Full list of author information is available at the end of the article

for atypical antipsychotics (Figure 1). These antipsychotics showed a regional preference for the mesolimbic dopamine receptors and also an optimum pK_i $5HT_{2A}/D_2$ binding affinity ratio [15]. Over the last decade, olanzapine with its thienobenzodiazepine core structure has been the first choice agent in the therapy of schizophrenia due to its high clinical efficacy [16]. However, treatment with olanzapine and the majority of the atypical antipsychotic agents is accompanied by excessive weight gain and severe metabolic side effects such as type II diabetes mellitus, hyperglycemia, dyslipidemia, and insulin resistance [1,3,17-20]. These antipsychotic drugs are liable for a complex of pathological changes in which not a single factor can be accountable for the induced metabolic side effects. Changes in hormonal peptide levels correlated with food intake (such as insulin and leptin) have been suggested to play a part in weight gain induced by atypical antipsychotic drug treatment [21]. Clozapine and olanzapine medications [22,23] in particular significantly influence the regulation of plasmatic insulin and leptin, although the mechanism of action remains elusive. A large number of studies have also reported a relevant role for the affinities of atypical antipsychotics for the $5HT_{2A}$, $5HT_6$, $5HT_7$, α_{1A}, and particularly H_1 and $5HT_{2C}$ receptors in their obesogenic effects [24-27].

There is much compelling evidence of the involvement of $5HT_{2C}$ receptors in satiety, food intake and body weight reviewed in [28]. It is reported that $5HT_{2C}$ antagonism or inverse agonism may contribute to olanzapine-induced weight gain [29]. A study on mice revealed that animals with selectively expressed $5HT_{2C}$ receptors only in POMC neurons are obese and hyperphagic, suggesting that POMC neurons mainly mediate the effects of $5HT_{2C}$ receptors on energy balance [30]. Moreover, previous findings presented evidence that $5HT_{2C}$ receptor and leptin gene polymorphisms, and smoking are imperative risk factors influencing the development of metabolic side effects in patient receiving antipsychotic drug treatments [31,32]. There are other receptors mechanism recognized with synergistic effect on weight gain and metabolic dysfunctions induced by antipsychotic drugs. D_2 receptor antagonism, for instance, can influence feeding behaviour [33], with an increase in $5HT_{2C}$-mediated effects on food intake. In addition, a disruption of rewarding mechanisms by D_2 receptor blockade in the dopamine reward system might results in a disinhibition of food intake; however, these effects are of less concern for the atypical antipsychotic drugs as their $5HT_{2A}$ antagonism proprieties attenuate the disruption of reward mechanisms by D_2 antagonism [27,34].

The blockade of the H_1 receptors has been repeatedly described as the most likely mechanism for atypical antipsychotic drug-induced weight gain [35-38]. The inhibition of the H_1 receptors is directly involved in the activation of hypothalamic AMPK signaling, which stimulates food intake and positive energy balance and reverses the anorexigenic effect of leptin [36]. A strong link between the H_1 receptor affinity of antipsychotic agents and their weight gain propensity has been reported [39]. Clozapine and olanzapine which have higher affinity for the H_1 receptors ($K_i = 1.2$ nM and $K_i = 2.0$ nM, respectively) showed a greater propensity to induce weight gain [40,41]. However, tetracyclic antipsychotic drugs with lower H_1 antagonist affinity, such as loxapine (**3**) and amoxapine (**4**) (Figure 1) caused neither weight gain nor weight loss in patients treated with these medications [42,43]. Thus, the development of a novel antipsychotic agent with similar $5HT_{2A}/D_2$ receptor binding affinity ratio to that of olanzapine and with a lower affinity to the H_1 receptors may significantly advance schizophrenia therapy. For the last several years, researches have been focused on developing novel antipsychotics with fewer metabolic side effects [44]. Nevertheless, the crucial needs for developing an ideal antipsychotic agent for schizophrenia still continue. In this study, we synthesized two novel derivatives of olanzapine (**8a** and **8b**) and the previously reported analogue **8c** [45], presenting two alterations to the structure of olanzapine such as the substitution of an ethyl group at the C-2 position of the thiophene ring (**8b** and **8c**) and the replacement of the *N*-methylpiperazine with an *N*-methylhomopiperazine ring (**8a** and **8b**) (Scheme 1). We then evaluated the affinities of these compounds for

Clozapine (**1**)

Olanzapine (**2**)

Loxapine (**3**)

Amoxapine (**4**)

**Figure 1 Structures of tetracyclic atypical antipsychotics.**

serotoninergic, dopaminergic, and histaminergic receptors in the prefrontal cortex, striatum, and hypothalamus respectively, which is relevant to their therapeutic and metabolic side effects.

Results

The syntheses of thienobenzodiazepines **8b** and **8c** were based on the procedure previously reported for the preparation of olanzapine by Shastri *et al.* [46] (Scheme 1) with some modifications. The condensation of 2-amino-5-ethylthiophene-3-carbonitrile (**5b**) with 1-fluoro-2-nitrobenzene followed by the intramolecular reductive cyclization of the thiophene-3-carbonitrile **6b** in the presence of stannous chloride led to amidine **7b** in 80% over 2 steps. This primary amidine was the key precursor for our two target compounds **8b** and **8c**. Treatment of amidine **7b** with *N*-methylpiperazine in conjugation with *N*-methylpiperazine hydrochloride salt in DMSO at 110 – 120°C afforded **8c** in 56% yield as bright yellow crystals. Interestingly, when amidine **7b** was reacted with *N*-methylhomopiperazine and *N*-methylhomopiperazine hydrochloride under the same conditions, **8b** was obtained in low yield (<20%) and purity due to the formation of many by-products, including the dimer of **8b**. Optimization of this step was therefore necessary for a

more efficient production of compound **8b**. After many explorations, we found that the reaction time to be the crucial factor in this process. Thus, to accelerate the reaction we applied microwave irradiation instead of conventional oil bath heating of the reaction mixture. This modification led to a reduction of the reaction time from 20 h to 3 h. Due to the shorter reaction time, the formation of the undesirable by-products was avoided and the yield of compound **8b** consequently increased. Furthermore, we found that the addition of *N*-methylhomopiperazine hydrochloride and a solvent was not necessary for the successful outcome of this reaction. When finally performed under solvent free conditions and microwave heating at 120°C for 3 h, then compound **8b** was obtained in an improved yield of 55%. The synthesis of compound **8a** required even milder reaction conditions to suppress side reactions. Thus, the microwave reaction of the commercially available amidine **7a** with *N*-methylhomopiperazine at 80°C for 4 h led to the formation of thienobenzodiazepine **8a** in 65% yield.

Next, we examined the receptor binding affinities of compounds **8a**, **8b** and **8c** for D_2, $5HT_{2A}$, and H_1 receptors in Sprague Dawley rats. The assays used were standard *in vitro* radioligand binding assays. Radioligand binding assays were run under the conditions described previously with some modifications [47,48]. The affinities of compound **8c** for the D_2 and $5HT_{2A}$ receptors were similar to the values found for olanzapine ($P = 0.561$ and $P = 0.959$, respectively) (Table 1). However, compound **8b** showed less binding affinities than olanzapine for D_2 and $5HT_{2A}$ receptors ($P = 0.002$ and $P = 0.030$, respectively). The pK_i ratios $5HT_{2A}/D_2$ of **8c** and **8b** were similar to the value of olanzapine ($P = 0.137$ and $P = 0.457$, respectively). This value ratio is consistent with the previously reported one for olanzapine in the literature [47,49]. Compounds **8a** displayed a significant reduction in binding to the D_2 and $5HT_{2A}$ receptors ($P = 0.001$ and $P = 0.001$, respectively) receptors. The binding affinity of **8c**, **8b**, and **8a** for H_1 receptors was
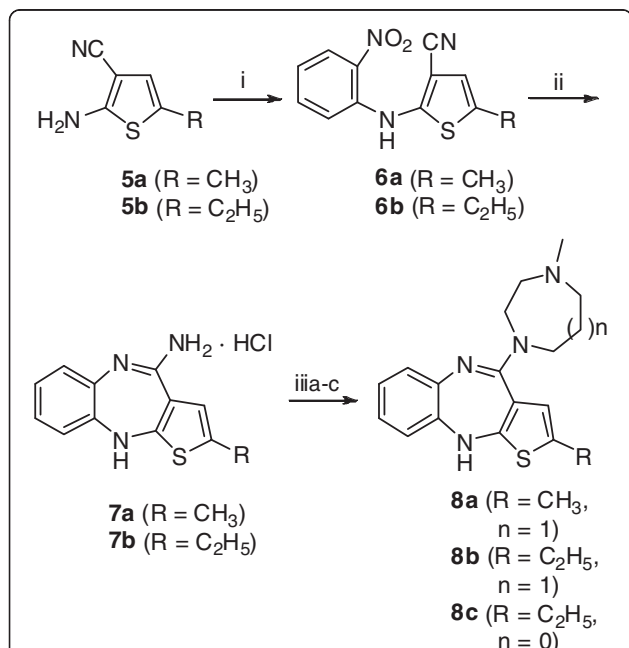
Scheme 1 Synthesis of compounds 8a, 8b, and 8c. Reagents and conditions: (i) 1-fluoro-2-nitrobenzene, NaH, THF, rt, 20 h, 60%; (ii) SnCl$_2$, EtOH, 80°C, 1 h, 80%; (iiia) *N*-methylhomopiperazine (5 equiv), no solvent, microwave heating, 80°C, 4 h,65%; (iiib) *N*-methylhomopiperazine (5 equiv), no solvent, microwave heating, 120°C, 3 h, 55%; (iiic) *N*-methylpiperazine (10 equiv), *N*-methylpiperazine hydrochloride (10 equiv), DMSO, 110–120°C, 20 h, 56%.

Table 1 Binding affinities of olanzapine, compounds 8a, 8b, and 8c for 5HT$_{2A}$, D$_2$, and H$_1$ receptors

Compound	$Ki \pm SD$ [a] (nM)			pK_i ratio $5HT_{2A}/D_2$
	D_2	$5HT_{2A}$	H_1	
olanzapine	67.72 ± 9.21	4.22 ± 0.77	0.13 ± 0.02	1.17
8c	54.51 ± 12.12	3.70 ± 0.74	1.95 ± 0.33*	1.16
8b	791.08 ± 83.19**	81.20 ± 2.43*	13.63 ± 2.68***	1.16
8a	3939.90 ± 437.35***	787.00 ± 29.81***	33.50 ± 7.39***	1.16

[a] Values are the mean ± SD of four to six experiments and represent the concentration giving half maximal inhibition of [^{3}H]-Spiperone (D$_2$), [^{3}H]-Ketanserine (5HT$_{2A}$), and [^{3}H]-Pyrilamine (H$_1$) binding to rat tissue homogenate. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs olanzapine values.

significantly reduced compared to olanzapine ($P = 0.033$, $P = 0.001$, and $P = 0.001$, respectively).

Discussion

Compound **8c** with an ethyl substituent at position 2 of the thiophene ring conferred a lower affinity for the H_1 receptors but similar affinities for the D_2 and $5HT_{2A}$ receptors when compared to olanzapine. This may suggest the potential contribution of a C-2 ethyl substituent to the potency of binding to the histaminergic receptors but not to the serotoninergic and dopaminergic receptors. On the other hand, the replacement of the *N*-methylpiperazine ring of compound **8c** with an *N*-methylhomopiperazine ring (**8b**) led to a reduction of the affinities for dopamine and serotonin receptors. This decrease of receptor affinities is probably caused by an unfavorable steric interaction of the *N*-methylhomopiperazine ring with amino acid residues in the binding pocket of the D_2 and $5HT_{2A}$ receptors. Lower affinity of compound **8b** for the H_1 receptors compared to olanzapine could be due to both the ethyl group and the *N*-methylhomopiperazine ring substitution. Although compound **8b** has lower affinity than olanzapine or compound **8c** for the D_2 and $5HT_{2A}$ receptors, it possessed a favorable pK_i ratio $5HT_{2A}/D_2$ similar to the measured value of olanzapine (ratio value = 1.17). Moreover, the obtained binding affinity of compound **8b** (Table 1) for the D_2 receptor is still comparable to that value of remoxipride (K_i: 800 nM) and quetiapine (K_i: 680 nM) [50], atypical antipsychotics with high therapeutic efficacy in the clinic. Therefore, it is assumed that the therapeutic effectiveness of compound **8b** may be delivered at higher doses than that of olanzapine administered. However, without appropriate comparison of DMPK (Drug Metabolism/Pharmacokinetics) properties between these mentioned antipsychotics (quetiapine, remoxipride, and olanzapine) and compound **8b**, the above notion could be an over-prediction. As a part of drug development process, it is crucial to investigate the preclinical toxicity and pharmacodynamics properties of the candidate drugs before risking and enhancing the possibility of further costly development.

Similarly, the presence of the *N*-methylhomopiperazine ring in thienobenzodiazepine **8a** caused a significant reduction in binding to the D_2 and $5HT_{2A}$ as well as the H_1 receptors. The low binding affinity of compound **8a** for the D_2 and $5HT_{2A}$ receptors may be due to a lower bioactive conformational state for the tricyclic thienobenzodiazepine skeleton. Steric effect of the *N*-methylhomopiperazine ring may also affect the rotation of this ring around the tricyclic thienobenzodiazepine, resulting in a lower interaction with the D_2 receptors in compounds **8a** and **8b**. However, the extension at the C-2 position of the thiophene ring with an ethyl group in compound **8b** seems to improve the affinities for the D_2

and $5HT_{2A}$ receptors when compared to those values of compound **8a**.

Taking these results into account, it appears that the *N*-methylhomopiperazine ring is not a favorable group for the interaction of the molecule with serotonin and dopamine receptors. The distances between the centroid of the fused thiophene and phenyl rings and the basic distal nitrogen as well as the conformational state of the tricyclic thienobenzodiazepine are essential parameters for the affinity of the molecule for the D_2 and $5HT_{2A}$ receptors [49]. Presumably, alteration in these distances in OlzHomo **8b** and MeHomo **8a** structures is involved in the lower affinity of these compounds for the D_2 and $5HT_{2A}$ receptors compared to olanzapine.

We suggest that both compounds **8b** and **8c** may present therapeutic effectiveness for treating schizophrenia. In addition, these compounds present a lower affinity for H_1 receptors, which may have reduced effects on weight gain and metabolic disorders than those reported with olanzapine. Nonetheless, given the potential safety-concerns of these new compounds and the previously reported inconsistencies of antipsychotic-induced weight gain shown in animal models and in clinic, the present results should be taken with a degree of caution. Clinical studies showed that weight gain is liable to occur with any antipsychotic drug treatment. Moreover, the extent to which these drugs cause weight gain and metabolic dysregulation vary according to the type of antipsychotics prescribed and inter-individual differences in response to treatment and susceptibility to development of metabolic syndromes; therefore, we suggested that only further comprehensive animal studies and clinical trials will reveal the predictive validity of therapeutic efficacy and metabolic side effects of these two compounds.

Conclusions

In summary, in this study we have synthesized olanzapine derivatives **8a**, **8b**, and **8c** and we have evaluated the affinities of these compounds for the brain $5HT_{2A}$, D_2, and H_1 receptors. Compound **8c** represents a potential antipsychotic agent characterized by a highly favorable binding profile at $5HT_{2A}$ and D_2 receptors, similar to olanzapine, as well as lower affinity for H_1 receptors. These results may suggest the potential role of a C-2 ethyl group in reducing binding affinity to the H_1 receptors. Compounds **8a** and **8b** with a bulky seven-membered ring presented lower binding affinities to all the tested receptors. Taking into account that the D_2 affinity of thienobenzodiazepine **8b** is still comparable with that value of the high potent antipsychotics, we suggest that both compounds **8b** and **8c** may present therapeutic effectiveness for treating schizophrenia. In addition, these compounds present a lower affinity for H_1 receptors, which may have reduced effects on weight gain and metabolic disorders

than those reported with olanzapine; however, given the fact that at the state-of-the-art the emergence of weight gain is possible following any antipsychotics treatment, we suggested that the present results be taken with caution. Only further animal studies and clinical trials will shed light on the therapeutic effectiveness and metabolic side effects of these two compounds.

Methods

Synthetic procedures

A solution of amidine hydrochloride **7b** (1.62 mmol, 455 mg), methyl piperazine (10 equiv, 16.2 mmol, 3300 mg) and methyl piperazine hydrochloride (16.2 mmol, 3400 mg) in DMSO (15 mL) was heated to110°C for 20 h (Figure 2). After cooling to room temperature the reaction mixture was diluted with dichloromethane and washed twice with 1 M aqueous HCl solution. The dark brown colored hydrochloride was separated and basified to pH 7.5 – 8.5, using 1 M aqueous NaOH solution. The attained solution was extracted with dichloromethane and washed twice with brine, then dried over $MgSO_4$ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography and then crystallized from acetonitrile to give the desired product (296 mg) as bright yellow crystals in 56% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.21 (t, 3H, $J = 7.5$ Hz, H_3C-2″), 1.97 (quin, 2H, $J = 5.7$ Hz, H_2C-6′), 2.39 (s, 3H, CH_3 at N-4′), 2.64 (m, 4H, H_2C-1″ & H_2C-5′), 2.71 (t, 2 H, $J = 4.5$ Hz, H_2C-3′), 3.68 (t, 2H, $J = 5.7$ Hz, H_2C-7′), 3.74 (t, 2H, $J = 4.5$ Hz, H_2C-2′), 5.02 (s, 1 H, HN-10), 6.35 (s, 1H, HC-3), 6.60 (dd, 1H, $J_1 = 1.3$ Hz, $J_2 = 7.8$ Hz, HC-9), 6.82

(dt, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-8), 6.94 (dt, 1H, $J_1 = 1.5$ Hz, $J_2 = 7.5$ Hz, HC-7), 7.00 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ [ppm]: 15.6 (CH_3, C-2″), 23.6 (CH_2, C-1″), 28.1 (CH_2, C-6′), 46.8 (CH_3, N-4′), 48.4 (CH_2, C-7′), 48.5 (CH_2, C-2′), 58.0 (CH_2, C-5′), 58.6 (CH_2, C-3′), 118.9 (CH, C-9), 120.0 (C_q, C-3a), 121.2 (CH, C-3), 122.8 (CH, C-8), 124.6 (CH, C-7), 127.9 (CH, C-6) 136.8 (C_q, C-2), 141.7 (C_q, C-5a), 142.3 (C_q, C-9a), 150.9 (C_q, C-10a), 157.1 (C_q, C-4). IR (neat) v_{max} [cm^{-1}]: 2967(w), 2930(w), 2916(w), 2850(w), 1584(vs), 1558(s), 1514(w), 1464(s), 1410(s), 1362(m), 1317(w), 1287(w), 1240(m), 1212(m) 1168(m), 1128(m), 1086(w), 1038(m), 1017(w), 963(w), 912(m), 864(w), 856(w), 837(w), 822(w), 810(w), 804(w), 755(m), 737(m), 722(m), 711(m). HRMS (ESI) calcd for $C_{18}H_{23}N_4S$ $[M + H]^+$ 327.1643, found 327.1642.

A mixture of amidine hydrochloride **7b** (0.34 mmol, 96 mg) and *N*-methylhomopiperazine (5 equiv, 1.71 mmol, 195 mg) was stirred at 120°C for 3 h under microwave heating in a sealed reactor tube (Figure 3). After cooling to room temperature the reaction mixture was diluted with dichloromethane (3 ml) and washed twice with saturated sodium bicarbonate solution. The organic phase was washed with brine, then dried over $MgSO_4$ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography to give the desired product as a brown powder (64 mg) in 55% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.21 (t, 3H, $J = 7.5$ Hz, H_3C-2″), 1.97 (quin, 2H, $J = 5.7$ Hz, H_2C-6′), 2.39 (s, 3H, CH_3 at N-4′), 2.64 (m, 4H, H_2C-1″ & H_2C-5′), 2.71 (t, 2H, $J = 4.5$ Hz, H_2C-3′), 3.68 (t, 2H,
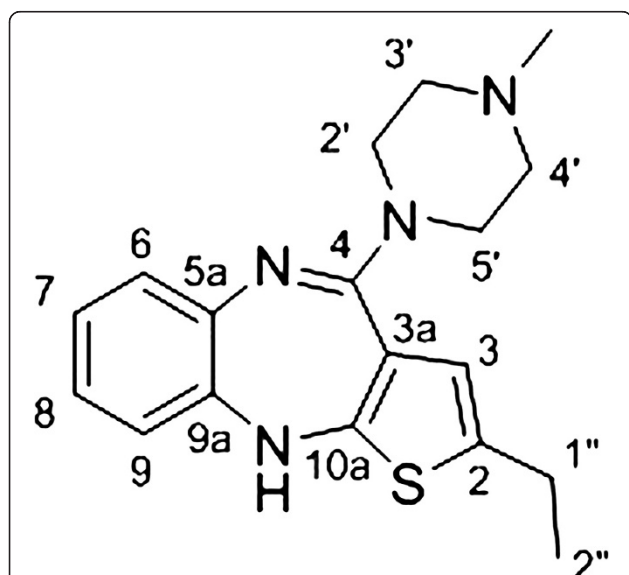
Figure 2 Procedure for the synthesis of compound (2-ethyl-4-(4'-methylpiperazin-1'-yl)-10Hbenzo[b]thieno[2,3-e][1,4] diazepine (8c).
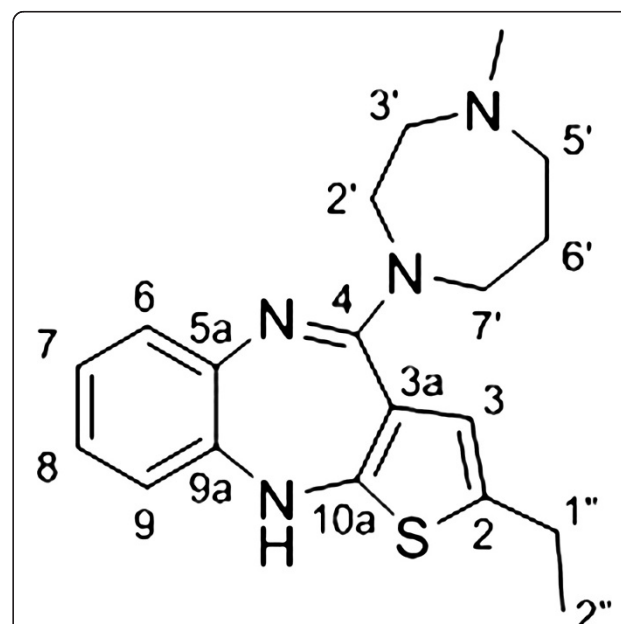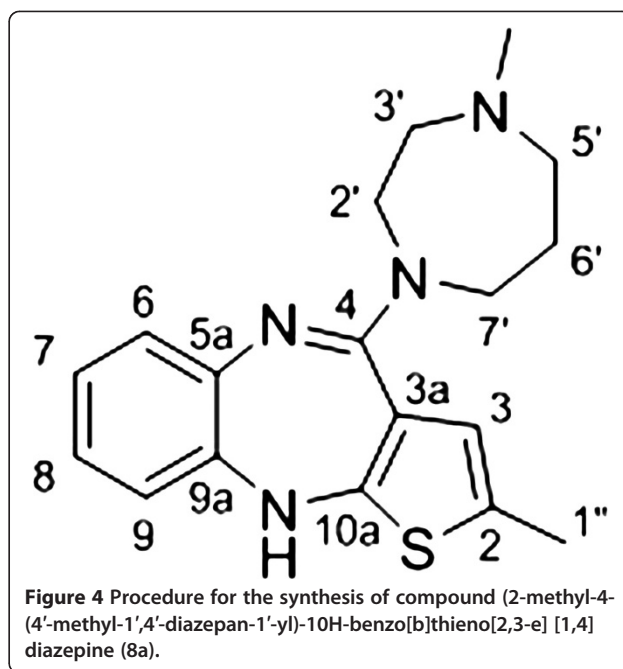

Figure 3 Procedure for the synthesis of compound (2-ethyl-4-(4'-methyl-1',4'-diazepan-1'-yl)-10H-benzo[b]thieno[2,3-e] [1,4] diazepine (8b).

$J = 5.7$ Hz, H_2C-7′), 3.74 (t, 2H, $J = 4.5$ Hz, H_2C-2′), 5.02 (s, 1H, HN-10), 6.35 (s, 1H, HC-3), 6.60 (dd, 1H, $J_1 = 1.3$ Hz, $J_2 = 7.8$ Hz, HC-9), 6.82 (dt, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-8), 6.94 (dt, 1H, $J_1 = 1.5$ Hz, $J_2 = 7.5$ Hz, HC-7), 7.00 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 7.8$ Hz, HC-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ [ppm]: 15.6 (CH_3, C-2″), 23.6 (CH_2, C-1″, 8.1 (CH_2, C-6′), 46.8 (CH_3, N-4′), 48.4 (CH_2, C-7′), 48.5 (CH_2, C-2′), 58.0 (CH_2, C-5′), 58.6 (CH_2, C-3′), 118.9 (CH, C-9), 120.0 (C_q, C-3a), 121.2 (CH, C-3), 122.8 (CH, C-8), 124.6 (CH, C-7), 127.9 (CH, C-6), 136.8 (C_q, C-2), 141.7 (C_q, C-5a), 142.3 (C_q, C-9a), 150.9 (C_q, C-10a), 157.1 (C_q, C-4). IR (neat) v_{max} [cm^{-1}]: 2940(w), 2850(w), 2803(w), 1583(vs), 1557(s), 1513(w), 1464(s), 1410(s), 1357 (m), 1317(w), 1288(m), 1247(m), 1196(m), 1017(w), 959(w), 910(m), 860(w), 848(w), 834(w), 830(w), 814(w), 753(vs), 740(s), 730(m), 717(w), 711(w), 702(w). HRMS (ESI) calcd for $C_{19}H_{25}N_4S$ $[M + H]^+$ 341.1800, found 341.1802.

A mixture of amidine hydrochloride **7a** (1 mmol, 260 mg) and *N*-methylhomopiperazine (5 equiv, 5 mmol, and 534 mg) was stirred at 80°C for 4 h under micro-wave heating in a sealed reactor tube (Figure 4). After cooling to room temperature the reaction mixture was diluted with dichloromethane (5 ml) and washed twice with saturated sodium bicarbonate solution. The organic phase was washed with brine, then dried over $MgSO_4$ and concentrated under reduced pressure to provide a brown oil. The crude product was purified by column chromatography to afford the desired product as a dark red powder (212 mg) in 65% yield. ^1H-NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.97 (quin, 2H, $J = 5.5$ Hz, H_2C-6′), 2.30 (s, 3H, H_3C-1″), 2.39 (s, 3H, CH_3 at N-4′), 2.65 (t, 2H, $J = 5.5$ Hz, H_2C-5′), 2.71 (t, 2H, $J = 4.5$ Hz, H_2C-3′), 3.68 (t, 2H, $J = 5.5$ Hz, H_2C-7′), 3.74 (s, 2H, H_2C-2′) 4.96 (s, 1H, HN-10), 6.33 (s, 1H, HC-3), 6.61 (d, 1H, $J = 7.5$ Hz, HC-9), 6.82 (t, 1H, $J = 7.5$ Hz, HC-8), 6.95 (t, 1H, $J = 7.5$ Hz, HC-7), 7.00 (d, 1H, $J = 7.5$ Hz, HC-6). ^{13}C-NMR (125 MHz, $CDCl_3$) δ [ppm]: 15.8 (CH_3, C-1″), 28.3 (CH_2, C-6′), 47.0 (CH_3, N-4′), 48.6 (CH_2, C-7′), 48.7 (CH_2, C-2′), 58.2 (CH_2, C-5′), 58.9 (CH_2, C-3′), 119.0 (CH, C-9), 120.5 (C_q, C-3a), 123.0 (CH, C-8), 123.2 (CH, C-3), 124.9 (CH, C-7), 128.1 (CH, C-6), 129.5 (C_q, C-2), 141.9 (C_q, C-5a), 142.4 (C_q, C-9a), 151.2 (C_q, C-10a), 157.1 (C_q,C-4). IR (neat) v_{max} [cm^{-1}]: 2940(w), 2850(w), 2803(w), 1583(vs), 1557(s), 1513(w), 1464(s), 1410(s), 1357(m), 1317(w), 1288(m), 1247(m), 1196(m), 1169(m), 1123(m), 1085(w), 1038(w), 1017(w), 959(w), 910(m), 860(w), 848(w), 834(w), 830(w), 814(w), 753(vs), 740(s), 730(m), 717(w), 711(w), 702(w). HRMS (ESI) calcd for $C_{18}H_{23}N_4S$ $[M + H^+]$ 327.1643, found 327.1642.

Membrane binding assay

The prefrontal cortex, striatum and hypothalamus were homogenized in about 6 volumes of ice-cold buffer Tris.HCl, 50 mM, pH 7.7 (for D_2 and $5HT_{2A}$ receptors) or Na^+/K^+

Figure 4 Procedure for the synthesis of compound (2-methyl-4-(4′-methyl-1′,4′-diazepan-1′-yl)-10H-benzo[b]thieno[2,3-e] [1,4] diazepine (8a).

phosphate 50 mM, pH 7.5 (for H_1 receptors), and the resultant homogenate was then centrifuged (27000 g for 15 min at 4°C). Each pellet was resuspended in the same volume of fresh buffer and centrifuged again under the same conditions as above. The final tissue pellets were resuspended just before the binding assay in the incubation buffer (50 mM Tris.HCl, 5 mM $MgSO_4$, 0.5 mM EDTA and 0.02% ascorbic acid, pH 7.7 for D_2 receptors; 50 mM Tris.HCl, pH 7.7 for $5HT_{2A}$ receptors and Na^+/K^+ phosphate 50 mM, pH 7.5 for H_1 receptors). $[^3H]$-Spiperone (specific activity, 15 Ci/mmol, 1 mCi/ml; Perkin Elmer, Australia) binding to D_2 receptor was assayed for 40 min at 37°C in the presence of 2 nM radioligand solution, 1.2 mg/mL protein suspension with/without serial concentration of displacers (olanzapine, **8a**, **8b**, and **8c**) The binding affinity at $5HT_{2A}$ receptors was measured in the presence of 10 nM $[^3H]$-Ketanserine (specific activity, 67 Ci/mmol, 1 mCi/ml; Perkin Elmer, Australia), protein suspension (0.25 mg/mL), with/without serial concentration of displacers. The reaction was carried out for 15 min at 37°C. $[^3H]$-Pyrilamine (specific activity, 37 Ci/mmol, 1 mCi/ml; Perkin Elmer, Australia) binding to H_1 receptor was assayed for 30 min at 25°C in the presence of radioligand solution (2 nM) with/without serial concentration of displacers. Incubation reactions were stopped by the addition of ice-cold buffer (Tris.HCl, 50 mM, pH 7.7 for D_2 and $5HT_{2A}$ receptors and Na^+/K^+ phosphate 50 mM for H_1 receptors) followed by rapid filtration on filter (GF/B Whatman). The filters were then washed twice with the buffer. The radioactivity on the filters was measured by beta liquid scintillation analyser (Perkin Elmer, Tri-Crab 2800 TR) with

a counting efficiency of 47%. IC_{50} values for olanzapine (Bosche Scientific) and compounds **8a**, **8b**, and **8c** were determined from competition curves and converted to K_i values using the Cheng-Prusoff equation [51]. Protein concentrations were determined by the Bradford method [52].

Statistical analysis

Data are presented as the means ± S.D. or averages. The significance of differences between two groups was evaluated using one-way ANOVA. The difference is considered as significant when the P value is less than 0.05.

Abbreviations
DMPK: Drug metabolism/pharmacokinetics; EPS: Extrapyramidal symptoms; 5-HT: 5-hydroxytryptamine (serotonin); TD: Tardive dyskinesia.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SJ designed the new compounds, carried out the organic synthesis, chemical analysis, radioligand binding assay, and the statistical analysis; participated in the design and coordination of the study and drafted the manuscript. MEB participated in the chemical analysis and organic synthesis and helped to draft the manuscript. XFH approved the manuscript and participated in the design and coordination of the study. SGP approved the chemistry part of the manuscript. FFE conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Author details
[1]Center for Translational Neurosciences, Illawarra Health and Medical Research Institute, School of Health Sciences, The University of Wollongong, Wollongong, NSW, Australia. [2]School of Chemistry, The University of Wollongong, Wollongong, NSW, Australia. [3]Schizophrenia Research Institute, Sydney, NSW, Australia.

References
1. Gardner DM, Baldessarini RJ, Waraich P: **Modern antipsychotic drugs: a critical overview.** *Synthese* 2005, **172**:1703–1711.
2. American Diabetes Association, American Psychiatric Association, American Association of Clinical Endocrinologist: **Consensus development conference on antipsychotic drugs and obesity and diabetes.** *Diabetes Care* 2004, **27**:596–601.
3. Dargham A, Laruelle M: **Mechanisms of action of second generation antipsychotic drugs in schizophrenia: insights from brain imaging studies.** *Eur Psychiatry* 2005, **20**:15–27.
4. Worrel JA, Marken PA, Beckman SE, Ruehter VL: **Atypical antipsychotic agents: a critical review.** *Am J Health Syst Pharm* 2000, **57**:238–255.
5. Seeman P, Lee T, Chau-Wong M, Wong K: **Antipsychotic drug doses and neuroleptic/dopamine receptors.** *Nature* 1976, **261**:717–719.
6. Serretti A, Ronchi DD, Lorenzi C, Berardi D: **New antipsychotics and schizophrenia: a review on efficacy and side effects.** *Curr Med Chem* 2004, **11**:343–358.
7. Bishara D, Taylor D: **Upcoming agents for the treatment of schizophrenia: mechanism of action, efficacy and tolerability.** *Drugs* 2008, **68**:2269–2292.
8. Conley RR, Kelly DL: **Second generation antipsychotics for schizophrenia: a review of clinical pharmacology and medication-associated side effects.** *J Psychiatry Relat Sci* 2005, **42**:51–60.
9. Oakley NR, Hayes AG, Sheehan MJ: **Effect of typical and atypical neuroleptics on the behavioural consequences of activation by muscimol of mesolimbic and nigro-striatal dopaminergic pathways in the rat.** *Psychopharmacology* 1991, **105**:204–208.
10. Tandon R, Jibson MD: **Extrapyramidal side effects of antipsychotic treatment: scope of problem and impact on outcome.** *Ann Clin Psychiatry* 2002, **14**:123–129.
11. Zhang Y, Xu H, He J, Yan B, Jiang W, Li X: **Quetiapine reverses altered locomotor activity and tyrosine hydroxylase immunoreactivity in rat caudate putamen following long-term haloperidol treatment.** *Neurosci Lett* 2007, **420**:66–71.
12. Casey DE: **The relationship of pharmacology to side effects.** *J Clin Psychiatry* 1997, **58**:55–62.
13. Meltzer HY, Matsubara S, Lee JC: **The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs.** *Psychopharmacol Bull* 1989, **25**:390–392.
14. Tehan BG, Lioyd EJ, Wong MG: **Molecular field analysis of clozapine analogs in the development of a pharmacophore model of antipsychotic drug action.** *J Mol Graph Model* 2001, **19**:418–426.
15. Buckley P: **Olanzapine: a critical review of recent literature.** *Expert Opin Pharmacother* 2005, **6**:2077–2089.
16. Allison DB, Mentore JL, Heo M, Chandler LP, Cappelleri JC: **Antipsychotic-induced weight gain: a comprehensive research synthesis.** *Am J Psychiatry* 1999, **156**:1686–1696.
17. Breden EL, Liu MT, Dean SR: **Metabolic and cardiacs side effects of second-generation antipsychotics: what every clinician should known.** *J Pharm Pract* 2009, **22**:478–488.
18. Holt RI: **Severe mental illness, antipsychotic drugs and the metabolic syndrome.** *The BJDVD* 2006, **6**:199–204.
19. Jones B, Basson BR, Walker DJ, Crawford MK, Kinon BJ: **Weight change and atypical antipsychotic treatment in patients with schizophrenia.** *J Clin Psychiatry* 2001, **62**:41–44.
20. Roerig JL, Steffen KJ, Mitchell JE, Crosby RD, Gosnell BA: **An exploration of the effect of modafinil on olanzapine associated weight gain in normal human subject.** *Biol Psychiatry* 2009, **65**:607–613.
21. Melkersson K: **Clozapine and olanzapine, but not conventional antipsychotics, increase insulin release in vitro.** *Eur Neuropsychopharmacol* 2004, **14**:115–119.
22. Bai YM, Chen TT, Yang WS, *et al*: **Association of adiponectin and metabolic syndrome among patients taking atypical antipsychotics for schizophrenia: a cohort study.** *Schizophr Res* 2009, **111**:1–8.
23. Reynolds GP, Zhang Z, Zhang X: **Association of antipsychotic drug induced weight gain with a 5-HT2C receptor gene polymorphism.** *Lancet* 2002, **359**:2086–2087.
24. Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Bryan L, Roth BL: **H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs.** *Neuropsychopharmacol* 2003, **28**:519–526.
25. Mathews M, Muzina JD: **Atypical antipsychotics: new drugs, new challenges.** *Clevel Clin J Med* 2007, **74**:597–606.
26. Reynolds GP, Kirk SL: **Metabolic side effect of antipsychotic treatment-pharmacological mechanism.** *Pharmacol Ther* 2010, **125**:169–179.
27. Coccurello R, Moles A: **Potential mechanism of atypical antipsychotic-induced metabolic derangement: clues for understanding obesity and novel drug design.** *Pharmacol Ther* 2010, **127**:210–251.
28. Kirk SL, Glazebrook J, Grayson B, Neill JC, Reynolds GP: **Olanzapine-induced weight gain in the rat: role of 5-HT2C and histamine H1 receptors.** *Psychopharmacology* 2009, **207**:119–125.
29. Xu Y, Jones JE, Kohno D, Williams KW, Lee CE, Choi MJ, *et al*: **5-HT2CRs expressed by pro-opiomelanocortin neurons regulate energy homeostasis.** *Neuron* 2008, **60**:582–589.
30. Yevtushenko OO, Cooper SJ, O'Neill R, Doherty JK, Woodside JW, Reynolds GP: **Influence of 5-HT2C receptor and leptin gene polymorphisms, smoking and drug treatment on metabolic disturbances in patients with schizophrenia.** *B J Psych* 2008, **192**:424–428.
31. Templeman LA, Reynolds GP, Arranz B, San L: **Polymorphisms of the 5-HT2C receptor and leptin genes are associated with antipsychotic drug-induced weight gain in Caucasian subjects with a first-episode psychosis.** *Pharmacogenet Genomics* 2005, **15**:195–200.
32. Clifton PG, Rusk IN, Cooper SJ: **Effects of dopamine D1 and dopamine D2 antagonists on the free feeding and drinking patterns of rats.** *Behav Neurosci* 1991, **105**:272–281.

33. Benaliouad F, Kapur S, Rompre PP: **Blockade of 5-HT2a receptors reduces haloperidol-induced attenuation of reward.** *Neuropsychopharmacology* 2007, **32**:551–561.
34. Matsui-Sakate A, Ohtani H, Sawada Y: **Receptor occupancy-based analysis of contribution of various receptors to antipsychotics-induced weight gain and diabetes mellitus.** *Drug Metab Pharmacokinet* 2005, **20**:368–378.
35. Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH: **Antipsychotic drug-induced weight gain mediated by histamine H_1 receptor-linked activation of hypothalamic AMP-kinase.** *PNAS* 2007, **104**:3456–3459.
36. Masaki T, Chiba S, Yasuda T, Noguchi H, Kakuma T, Watanabe T, Sakata T, Yoshimatsu H: **Involvement of hypothalamic histamine H_1 receptor in the regulation of feeding rhythm and obesity.** *Diabetes* 2004, **53**:2250–2260.
37. Masaki T, Yoshimatsu H: **The hypothalamic H_1 receptor: a novel therapeutic target for disrupting diurnal feeding rhythm and obesity.** *Trends Pharmacol Sci* 2006, **27**:279–284.
38. Mercer LP: **Histamine and the neuroregulation of food intake.** *Nutrition* 1997, **13**:581–582.
39. Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, Jayathilake K, Meltzer HY, Bryan L, Roth BL: **H_1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs.** *Neuropsychopharmacology* 2003, **28**:519–526.
40. Mercer LP, Kelley DS, Haq AU, Humphries LL: **Dietary induced anorexia: a review of involvement of the histaminergic system.** *J Am Coll Nutr* 1996, **15**:223–230.
41. Pijl H, Meinders AE: **Body weight change and adverse effect of drug treatment. Echansm and management.** *Drug Saf* 1996, **14**:329–342.
42. Recasens C: **Body weight changes and psychotropic drug treatment: neuroleptics.** *Encephale* 2001, **27**:269–276.
43. Jafari S, Fernandez-Enright F, Huang X-F: **Structural contributions of antipsychotic drugs to their therapeutic profiles and metabolic side effects.** *J Neurochem* 2012, **120**:371–384.
44. Chakrabarti JK, Horsman L, Hotten TM, Pullar IA, Tupper DE, Wright FC: **4-piperazinyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepines as potential neuroleptics.** *J Med Chem* 1980, **23**:878–884.
45. Shastri JA, Bhatnagar A, Thaper RK, Dubey SK: *Process for Producing Pure Form of 2-Methyl-4-(4-Methyl-1-Piperazinyl)-10H-Thiono[2,3-b][1,5] Benzodiazepine.* U.S. Patent 2006, WO 2006006180 A1 20060119.
46. Campiani G, Butini S, Fattorusso C, Catalanotti B, Gemma S, Nacci V, Morelli E, Cagnotto A, Mereghetti I, Mennini T, *et al*: **Pyrrolo[1,3]benzothiazepine-based serotonin and dopamine receptor antagonists. Molecular modeling, further structure-activity relationship studies, and identification of novel atypical antipsychotic agents.** *J Med Chem* 2004, **47**:143–157.
47. Durand M, Aguerre S, Fernandez F, Edno L, Combourieu I, Mormède P, Chaouloff F: **Strain-dependent neurochemical and neuroendocrine effects of desipramine, but not fluoxetine or imipramine, in spontaneously hypertensive and Wistar–Kyoto rats.** *Neuropharmacology* 2000, **39**:2464–2477.
48. Campiani G, Butini S, Fattorusso C, Trotta F, Gemma S, Catalanotti B, Nacci V, Fiorini I, Cagnotto A, Mereghetti I, *et al*: **Novel atypical antipsychotic agents: rational design, an efficient palladium-catalyzed route, and pharmacological studies.** *J Med Chem* 2005, **48**:1705–1708.
49. Seeman P: **Dopamine D_2 receptors as treatment target in schizophrenia.** *Clin Schizophr Relat Psychoses* 2010, **4**:56–73.
50. Cheng Y, Prusoff WH: **Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction.** *Biochem Pharmacol* 1973, **22**:3099–3108.
51. Bradford MM: **Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.** *Anal Biochem* 1976, **72**:248–254.