Novel Atypical Antipsychotic Agents: Rational Design, an Efficient Palladium-Catalyzed Route, and Pharmacological Studies

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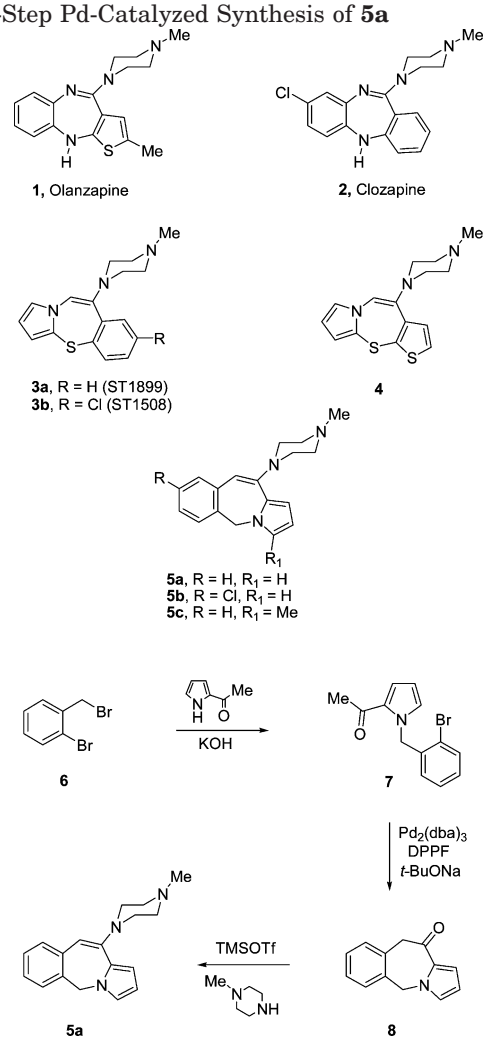
Abstract: Using rational drug design to develop atypical antipsychotic drug candidates, we generated novel and metabolically stable pyrrolobenzazepines with an optimized pK_i 5-HT_{2A}/D_2 ratio. **5a**, obtained by a new palladium-catalyzed three-step synthesis, was selected for further pharmacological and biochemical investigations and showed atypical antipsychotic properties in vivo. **5a** was active on conditioned avoidance response at 0.56 mg/kg, it had low cataleptic potential and proved to be better than ST1899, clozapine, and olanzapine, representing a new clinical candidate.

Schizophrenia is a disabling disease that affects 1% of the world population and is the second major psychiatric disease.[1] There are no specific characteristics for the diagnosis of schizophrenia, and no single symptom is consistently present in all patients.[2] The symptoms most commonly associated with the disease are positive symptoms and denote the presence of grossly abnormal behavior. These include thought disorder, delusions, and hallucinations. Less obvious than the positive symptoms but equally serious are the negative symptoms, namely, absence of normal behavior.[2] These include the flat or blunted affect (i.e., lack of emotional expression), apathy, and social withdrawal. Cognitive deficits include reduced working memory, attention, and verbal fluency. Before the 1990s, antipsychotic drug development focused exclusively on agents with substantial activity on dopamine receptors (typically haloperidol).[3] Typical antipsychotic drugs carry a heavy side effect burden, fail to manage negative symptoms (haloperidol), and are ineffective in about one-third of patients with schizophrenia. Over the past decade, the development of new antipsychotic agents has shifted from selective dopamine antagonist to compounds that have a broader receptor affinity profile (the so-called atypical antipsychotics), characterized by improved clinical efficacy and fewer side effects.[4] The first atypical neuroleptic drug was clozapine, followed by olanzapine (**1** and **2**, Chart 1). Although many atypical antipsychotic drugs have recently been approved for the treatment of schizophrenia,[5] olanzapine is still invaluable for psychosis, having high clinical efficacy. However, olanzapine may precipitate or unmask diabetes in susceptible patients, and it has been associated with a 12% increase in excessive appetite with respect to haloperidol.[6]

In the past 10 years, several novel strategies (glutamate (mGluRs)[2] and tachykinin receptors)[7] have been proposed for the development of atypical antipsychotics with fewer side effects; nevertheless, the need for an ideal antipsychotic agent continues to stimulate the search for newer and safer drugs. We recently developed a new class of atypical antipsychotics with the pyrrolo-[2,1-*b*][1,3]benzothiazepine structure.[8] The benzothiazepine **3a** (ST1899) (Chart 1) showed an atypical binding profile, and in vivo pharmacological studies indicated its pharmacological behavior to be superior to that of

Chart 1. Reference and Title Compounds and the Three-Step Pd-Catalyzed Synthesis of **5a**



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olanzapine. However, **3a** may undergo several metabolic pathways in vivo, including the oxidative transformation into its sulfoxide derivative, that could lead to a partial inactivation, as shown for quetiapine[9] and octoclothepin.

To provide drug therapies with prompter therapeutic benefit for "resistant" schizophrenic patients, we decided to investigate novel tricyclic systems with the aim of developing an atypical drug candidate characterized by improved efficacy (**4** and **5a**−**c**) and, presumably, metabolic stability (**5a**−**c**). Here, we describe the design, synthesis, and biological evaluation of novel benzazepine antipsychotics, characterized by absence of the sulfur-bridged atom of **3a**,**b** sensitive to oxidative metabolism, and their SARs for dopamine and serotonin receptor affinity. Among the analogues synthesized and tested, **5a** was selected for further biological investigation. We discuss its development and a molecular modeling study.

The set of compounds included in the molecular modeling study was synthesized as described in Schemes 1 and 2 in the Supporting Information. The main goal in the synthesis of the principal compound of the series, **5a**, was the newly developed palladium-catalyzed three-step pathway that provided the desired benzazepine with high overall yield (Chart 1). The new tricyclic compounds were subjected to binding experiments to evaluate their affinity for rat serotonin and dopamine receptor subtypes, and **5a** was selected for thorough investigation in vivo.

In a previous study, we demonstrated the possibility of modulating the in vivo properties (typical/atypical) of new potential tricyclic antipsychotics by specifically adapting their potency toward D_2 receptors.[9a−c] Our strategy was based on the hypothesis that tricyclic antipsychotics may interact with the D_2 receptor active site by adopting both folds of the tricyclic system and that the chemical/physical properties of the tricyclic skeleton can drive the binding mode determining which aromatic ring is preferentially recognized as the "relevant" one. To expand our SAR studies and to obtain molecules with superior antipsychotic efficacy, we used this hypothesis in designing the **4** and **5a**−**c** (Chart 1), based on a pyrrolo[2,1-*b*]thieno[3,2-*f*][1,3]thiazepine and a pyrrolo[1,2-*b*][2]benzazepine skeleton, respectively. Since early benzothiazepines, represented by **3a**, may undergo oxidative metabolism in vivo at the bridged-sulfur atom and, as in the case of quetiapine and octoclothepin, the oxidized metabolite may have weak to negligible antipsychotic activity,[9] the pyrrolo[1,2-*b*]-[2]benzazepine system (**5a**−**c**) was investigated to obtain potential antipsychotics characterized by higher metabolic stability due to absence of the bridged-sulfur atom. Calculated structural parameters reported in Table 3 (Supporting Information) show that a butterfly-like conformation of the tricyclic system, optimal for D_2 receptor interaction,[8] is maintained in both skeletons, as confirmed by the X-ray structure of **5a** (Figure 2, Supporting Information). Starting from lead **3a**, we developed **4** and **5a**−**c** rationally modifying several structural features that can specifically affect D_2 receptor affinity, such as the distance between the centroid of the relevant aromatic ring and the basic nitrogen. To test the two possible binding modes for the new compounds at the D_2 receptor,[8] we calculated the conformational inversion energy barrier for the tricyclic system of **5a**−**c**, which was around 14 and 16 kcal/mol, using MM (Discover, Insight2000.1, Accelrys, San Diego) and semiempirical (MOPAC/PM3) optimization methods, respectively. Neither the chlorine substituent of **5b** nor the methyl substituent of **5c** affected the energy inversion barrier values. On the other hand, the pyrrolo[2,1-*b*]thieno[3,2-*f*][1,3]thiazepine system of **4** showed a lower conformational inversion energy barrier (6.4 and 11.0 kcal/mol in MM and PM3 calculations, respectively). Taken together, these results suggested that the pyrrolo[1,2-*b*][2]benzazepine and pyrrolo[2,1-*b*]thieno[3,2-*f*][1,3]thiazepine systems can undergo ring inversion under physiological conditions, as happens for many other tricyclic systems,[9c] resulting in A-fold and B-fold types (negative and positive values of τ, respectively; Table 3 in Supporting Information). We then did a comprehensive conformational search, systematically varying all rotatable bonds, to determine the corresponding energy minima. The τ_N value required for positioning the lone pair of the distal piperazine nitrogen to optimally fulfill the D_2 pharmacophore was established for both folds (BIO piperazine position; Table 4 in Supporting Information). The resulting MM conformers, as well as the BIO conformations, were used as starting points for full semiempirical PM3 geometry optimization. The first major result was that the BIO conformations of **5a**−**c** showed an increased energy difference from the global minimum ($\Delta E_{GM} \cong 3.0$ kcal/mol; Table 4 in Supporting Information) with respect to benzothiazepine-based derivatives such as **3a** ($\Delta E_{GM} \cong 1.8$ kcal/mol in PM3 calculations[8]). This could be explained by the following considerations. The pyrrolo-[2,1-*b*][1,3]benzothiazepine system of **3a**,**b** and the pyrrolo[1,2-*b*][2]benzazepine of **5a**−**c** show the pyrrole nitrogen at two different positions. In **3a**,**b** the nitrogen is closer to the bridged double bond (C9−C10) with respect to the heterocyclic core system of **5a**−**c**, and this fact affects electronic conjugation between the vicinal piperazine nitrogen (N1) and the bridged double bond. The decreased degree of electronic conjugation found on the **5a**−**c** core system rendered the D_2 bioactive conformation (BIO piperazine position) energetically disfavored. Accordingly, the BIO conformations of **4**, in which the pyrrole nitrogen is closer to the bridged double bond, showed decreased conformational energies (~2.4 kcal/mol; Table 4 in Supporting Information). This hypothesis is further supported by the analysis of charge distribution on the PM3 optimized bioactive conformations of **3a**, **4**, and **5a**−**c** and by τ_N values reported in Table 4. Consequently, **5a**−**c** were predicted to be on the whole less potent at D_2 receptors than benzothiazepines. Our calculations showed that the BIO piperazine position is equally energetically accessible for both folds of the tricyclic systems of **4** and **5a**−**c** (Figure 1; Table 4 in Supporting Information).

In Figure 1 is reported the fit between the D_2 receptor 3D pharmacophore and the newly developed compounds, considering either the A- or the B-fold of the tricyclic system, compared to the binding mode of the reference **3b** ($K_{i-D2} = 0.43$ nM[8]). When **5b** binds D_2 receptor adopting an A-fold, the chlorine atom favorably interacts with the relevant polarized aromatic ring

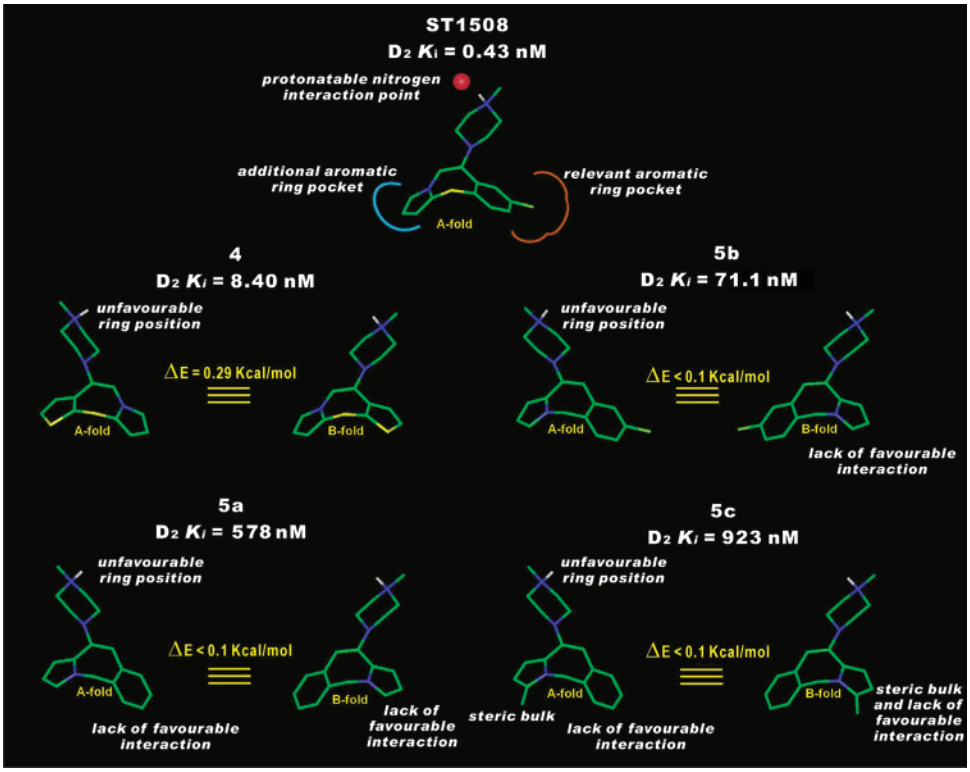

Figure 1. Hypothetical D_2 binding modes for **3b** (ST1508), **4**, and **5a**−**c**. Structures are superimposed on the centroids of the aromatic rings and a point 2.8 Å along the nitrogen lone pair vector. Nitrogens are in blue, carbons in green, chlorines in light-green, sulfurs in yellow. Hydrogens were omitted except for the one protonating the distal nitrogen (white). $\Delta E = E_{A-fold} - E_{B-fold}$.

Table 1. Binding Affinities for 5-HT_{2A}, 5-HT_{2C}, D_1, D_2, and D_3 Receptors of **4** and **5a**−**c**, and Reference Compounds

compd	R	R_1	$K_i \pm$ SD,[a] nM 5-HT_{2A}	D_1	D_2	D_3	5-HT_{2C}	pK_i ratio 5-HT_{2A}/D_2	log Y [b]
4			2.15 ± 0.32	4.91 ± 0.5	8.40 ± 1.0	25.0 ± 3.9	-	1.07	6.70
5a	H	H	7.25 ± 1.2	125 ± 12	578 ± 46	224 ± 23	-	1.30	3.20
5b	Cl	H	1.35 ± 0.08	47.4 ± 2.6	71.1± 12	24.2 ± 2.7	3.8 ± 0.35	1.24	3.91
5c	H	Me	4.99 ± 1.1	230 ± 18	923 ± 306	88.6 ± 23	15.0 ± 1.2	1.38	2.33
1, olanzapine			4.0 ± 1.0	85.0 ± 3.5	69.0 ± 17.0	39.0 ± 5.9	-	1.17	4.69
2, clozapine			10.0 ± 1.0	353 ± 35.0	250 ± 57.0	319 ± 45.0	-	1.21	3.89
3a, ST1899[c]			0.65 ± 0.1	19.7 ± 1.3	17.2 ± 4.5	8.30 ± 0.5	-	1.18	4.98
haloperidol			164 ± 22.0	318 ± 59.0	4.80 ± 1.0	18.0 ± 1.5	-	0.82	9.14

[a] Values are the mean ± SD of three determinations and indicate the concentration giving half-maximal inhibition of [^{3}H]ketanserin (5-HT_{2A}), [^{3}H]SCH 23390 (D_1), [^{3}H]spiperone (D_2), and [^{3}H]mesulergine (5-HT_{2C}) binding to rat tissue homogenate and [^{3}H]-7-OH-DPAT (D_3) binding to Sf9 cell membranes. [b] The log Y score was calculated as in ref 8. [c] From ref 8.

pocket of the receptor but the piperazine ring is unfavorably positioned; on the other hand, adopting the B-fold, **5b** is able to optimally orientate the piperazine ring but completely lacks a favorable interaction with the relevant aromatic ring pocket. Indeed, the dipole determined by the fused pyrrole ring of **5b** produced a vector perpendicular to that given by the dipole of the chloro-substituted benzo-fused system (Decipher, Insight2000.1, Accelrys). Adopting both folds, **5b** (K_{i-D2} = 71.1 nM, Table 1) could not optimally fulfill the D_2 pharmacophore, as occurs for **3b** (K_{i-D2} = 0.43 nM; Figure 1). When the chlorine atom of **5b** is replaced by a hydrogen, the resulting **5a** lost another D_2 receptor interaction point and **5a** was predicted to have a lower D_2 receptor affinity (**5a**, K_{i-D2} = 578 nM; **5b**, K_{i-D2} = 71.1 nM). Taking into account previous 3D-SAR studies on a series of antipsychotics,[9c] we introduced a methyl substituent at C1 of the pyrrole ring. As reported in Figure 1, the resulting **5c** has further unfavorable features, assuming either an A- or B-fold (**5c**, K_{i-D2} = 923 nM). On the other hand, when the benzo-fused ring of **3a** is replaced by an isosteric thiophene ring (**4**), the polarization of thiophene (Decipher, Insight2000.1, Accelrys) is still favorable for D_2 receptor interaction, mimicking the chloro-substituted ring of **3b**. As a consequence, adopting a B-fold, **4** accommodates the piperazine ring in the right orientation, still keeping favorable $\pi-\pi$ interactions with the relevant aromatic ring pocket of the receptor (Figure 1). In line with our hypothesis, **4** showed an increased D_2 receptor affinity (**4**, K_{i-D2} = 8.4 nM). **5a** was also tested on 5-HT_{2A} recombinant human receptors ([^{3}H]ketanserin) and showed a K_i of 5.4 nM.

Benzazepine **5a** was identified as a potential atypical antipsychotic agent, and its pharmacological profile was confirmed in vivo. Tests were performed using **5a** as a free base, using the same experimental conditions as for clozapine, olanzapine, and **3a**.[8]

Blockade of spontaneous locomotor activity, antagonism of climbing elicited by the direct dopamine agonist apomorphine in mice, and reduction of conditioned avoidance response (CAR) in rats are robust and reproducible models, sensitive to D_2 receptor antagonists, for predicting therapeutic efficacy against positive symp-

Table 2. In Vivo Pharmacological Profile of **5a** and Inhibition of Different Behavioral Responses after Oral Administration of the Test and Reference Compounds

	5-MeO-DMT-induced head twitches		apomorphine climbing		spontaneous locomotor activity		CAR		CAT	
compd	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]
1, olanzapine[b]	1.45	4.65	2.69	8.61	2.81	8.99	1.46	4.67	21.2	67.9
2, clozapine[b]	5.35	16.4	5.72	17.5	5.50	16.8	4.89	15.0	>100	>306
3a, ST1899[b]	1.99	6.69	1.64	5.51	0.51	1.71	1.09	3.66	>100	>336.2
5a	0.12	0.43	0.14	0.50	1.60	5.73	0.56	2.00	>100	>357.4 (>179[c])

[a] Results are expressed as ED_{50}. [b] From ref 8. [c] CAT/CAR for **5a**.

toms. These behavioral assays have been employed in vivo to demonstrate **5a** dopamine antagonist activity and indirectly its ability to interact with the mesolimbic dopaminergic system, as predictive of **5a** antipsychotic potential. Rat spontaneous locomotor activity was significantly reduced by **5a**, with $ID_{50} = 1.60$ mg/kg (Table 2). The ability to antagonize apomorphine-induced climbing behavior in mice was also evaluated. Oral **5a**, prior to 1.3 mg/kg apomorphine challenge, caused dose-related suppression of apomorphine-induced climbing behavior (Table 2) with $ED_{50} = 0.50$ μmol/kg, much lower than **3a** ($ED_{50} = 5.5$ μmol/kg), olanzapine ($ED_{50} = 8.61$ μmol/kg), and clozapine ($ED_{50} = 17.5$ μmol/kg). In light of these results, **5a** was selected for further studies.

The antipsychotic potential of **5a** was measured by evaluation of the CAR in rats. In the shuttle-box test, oral **5a** suppressed CAR with no significant effects on escape responses (data not shown). As shown in Table 2, **5a** was more potent than clozapine, olanzapine, and **3a** (ED_{50}: **5a**, 2.0 μmol/kg; clozapine, 15 μmol/kg; olanzapine, 4.67 μmol/kg; **3a**, 3.69 μmol/kg). Furthermore, **5a** antagonizes 5-HT_{2A} receptors in vivo. After sc injection (6 min) of 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT, 10 mg/kg, sc), we measured the number of 5-MeO-DMT-induced head twitches for 15 min. **5a** was administered orally 60 min before 5-MeO-DMT, and the data were compared with clozapine, olanzapine, and **3a**. **5a** antagonized 5-MeO-DMT-induced head twitches at doses much lower than that of olanzapine ($ED_{50} =$ 0.43 and 4.65 μmol/kg, respectively). Higher doses of clozapine and **3a** were necessary to achieve the same effect (ED_{50}: **3a**, 6.7 μmol/kg; **2**, 16.4 μmol/kg). **5a** also has a limited propensity to elicit catalepsy (CAT). The degree of catalepsy is often used to predict the incidence of extrapyramidal motor disorders. **5a** was administered orally to rats at doses of 100 mg/kg, and the catalepsy response was evaluated after 60, 120, 180, 240, and 300 min. Like clozapine (>100 mg/kg, >306 μmol/kg, po) but unlike olanzapine, which under the same experimental conditions induced catalepsy in 50% of animals 240 min after oral administration of 21 mg/kg ($ED_{50} = 67.9$ μmol/kg),[8] **5a** had low cataleptogenic potential (ED_{50}: >100 mg/kg, >357.4 μmol/kg). Thus, compared to olanzapine, **5a** has a higher threshold for inducing catalepsy that may by analogy translate into lower clinical EPS liability. This limited propensity to elicit catalepsy results in a large spread between doses, possibly inducing catalepsy and blocking the avoidance response (CAT/CAR > 179). These results suggest a preferential ability of **5a** to modulate mesolimbic instead of nigrostriatal dopaminergic neurotransmission, highlighting its atypicality and low propensity to induce unwanted extrapyramidal motor disturbances at therapeutically useful doses.

In conclusion, these results demonstrate the rational design of the novel and highly potent atypical antipsychotic agent **5a** (ST2329). Its receptor affinity profile suggests a complex interaction with cortical receptors involved in regulation of the activity of prefrontal cortical cells innervated by ventral tegmental area neurons, like 5-HT_{2A}, and dopaminergic receptor subtypes. The 5-HT_{2A}/D_2 ratio (expressed by $\log Y = 3.20$) is particularly favorable, and preliminary in vivo studies confirmed pharmacological effects superior to those of olanzapine and clozapine on 5-OMe-DMT-induced head twitches, apomorphine-induced climbing, and spontaneous locomotor activity in animals, CAT, and CAR. **5a** displayed atypical antipsychotic activity at 0.56 mg/kg, an oral dose much lower than that of olanzapine and **3a**. The atypical antipsychotic **5a**, characterized by improved efficacy and better metabolic stability than **3a** and olanzapine and obtained by an elegant three-step synthesis, was selected for thorough pharmacological investigation as a potential clinical candidate.

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Supporting Information Available: Experimental details for **4** and **5a**−**c**, Tables 3 and 4, Figure 2, and Schemes 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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