A Mutual Prodrug Ester of GABA and Perphenazine Exhibits Antischizophrenic Efficacy with Diminished Extrapyramidal Effects

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Received October 2, 2007

The perphenazine and fluphenazine GABA esters **3** and **4** evaluated in rat models for antipsychotic activity displayed a significant decrease of catalepsy associated with increased prolactin blood levels. Efficacy was evaluated in the D-amphetamine-induced hyperactivity model, where perphenazine abolished hyperactivity and induced sedation and catalepsy, whereas **3** reduced hyperactivity without sedation or catalepsy. Thus, **3** (**BL-1020**) constitutes a prototype of novel antipsychotics possessing GABAergic activity. A phase II study is in progress.

Introduction

Neuroleptics are drugs widely used in the treatment of schizophrenia and related psychotic disorders. Typical drugs such as the phenothiazines or haloperidol are potent inducers of extrapyramidal symptoms (EPS).[a,1,2] About 5% of the patients on chronic therapy develop pathologic tardive dyskinesia.[3,4] The most widely considered neurochemical hypothesis of schizophrenia is the dopamine hypothesis, which postulates that symptoms of schizophrenia result from an imbalance in dopaminergic D1 and D2 receptors.[5] In addition, decreased activity of the γ-aminobutyric acid (GABA) system in the brain contributes to the pathology of the disease.[6] An alternative neurochemical model of schizophrenia has been proposed to involve glutamatergic mechanisms, supported by increasing evidence of dysfunction in glutamate and GABA as well as dopamine systems.[7]

In line with the hypothesis that increased dopamine release and sensitization of the dopaminergic system are the major causes of schizophrenic manifestations,[5,8] most approved antipsychotics target dopamine receptors.[6,9] First-generation, typical antipsychotic drugs such as perphenazine, fluphenazine, and haloperidol are potent antagonists of dopamine receptors, especially D_2. The drawbacks of these drugs are EPS and the development of dopamine receptor hypersensitization in the basal ganglia.[1,2,10]

Atypical agents such as clozapine, olanzapine, and risperidone, which possess lower affinity to dopamine D2 receptors and induce antiserotonin activity, also display adverse side effects involving metabolic disturbances leading to increase in body weight, diabetes, changes in mood, sexual dysfunction, and agranulocytosis.[11,12]

GABA, the major inhibitory neurotransmitter in the brain, possesses anticonvulsant, anxiolytic, mood-stabilizing, hypnotic, and muscle relaxant properties.[13] Postmortem studies have shown a GABAergic deficit in the brain of schizophrenic patients.[14–17] Abnormalities in GABA metabolism include reduced levels of GABA and decreased activity of an isoform of glutamic acid decarboxylase (GAD), which is the rate-limiting enzyme in the conversion of glutamate to GABA. Although it has been reported that provision of GABA ameliorates cognitive deficits of schizophrenia and mitigates EPS associated with dopamine blockade,[1,18,19] clinical treatment of schizophrenia with GABA is impractical because under normal conditions GABA does not transverse the blood−brain barrier (BBB).[20] It has been reported that conjugation of GABA with fatty amino acids or peptides facilitates its passage across the BBB.[21] Combined treatment of neuroleptics (e.g., dopamine antagonists) and GABAergic agonists (e.g., benzodiazepines) given to schizophrenic patients resulted in reduced EPS.[17,22–25] Previous investigations suggest that GABA agonists can interfere with brain neurotransmitters, in particular, the dopamine (DA) system, and antagonize neuroleptic-induced increase in DA receptor sensitivity and, therefore, can potentially improve neuroleptic-induced EPS or dyskinesia.[18]

The objective of these studies was to demonstrate the neuroleptic efficacy, oral bioavailability, and reduced EPS of novel GABA esters of the phenothiazines perphenazine and fluphenazine as compared to the parent molecules, where it was expected that the esters would cross the BBB and upon hydrolysis in the brain would concomitantly release GABA and the antipsychotic phenothiazines.

Results and Discussion

Chemistry. The synthesis of the GABA-phenothiazine esters is shown in Scheme 1. Various conditions for coupling Boc-GABA to perphenazine or fluphenazine were evaluated including activation of the Boc-GABA with carbonyldiimidazole (CDI), pivaloyl chloride, or dicyclohexylcarbodiimide (DCC). The CDI procedure gave somewhat low yields, and using pivaloyl chloride the obtained product was contaminated with some perphenazine pivalate. Thus, the most efficient procedure found for the synthesis of **1** and **2** was that involving DCC. Upon removal of the Boc protective group under acidic conditions, the corresponding salts of the esters were isolated. Since the trihydrochloride salt of **3** was found to be hygroscopic, other salts were prepared and examined. The trimaleate salt was found to be much less hygroscopic than the trihydrochloride; however, in the synthetic course involving initial Boc removal with trifluoroacetic acid followed by rapid neutralization with $NaHCO_3$ and final addition of 3 equiv of maleic acid (see Scheme 2), the trimaleate salt obtained was contaminated with a

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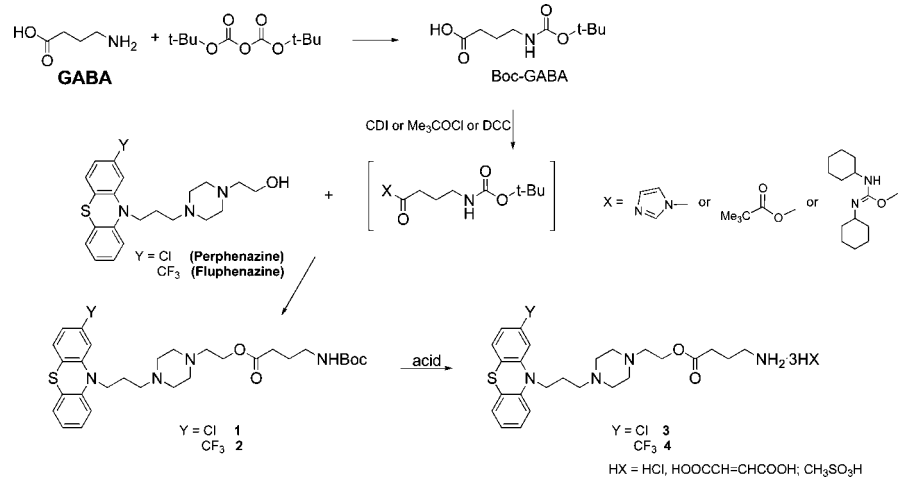
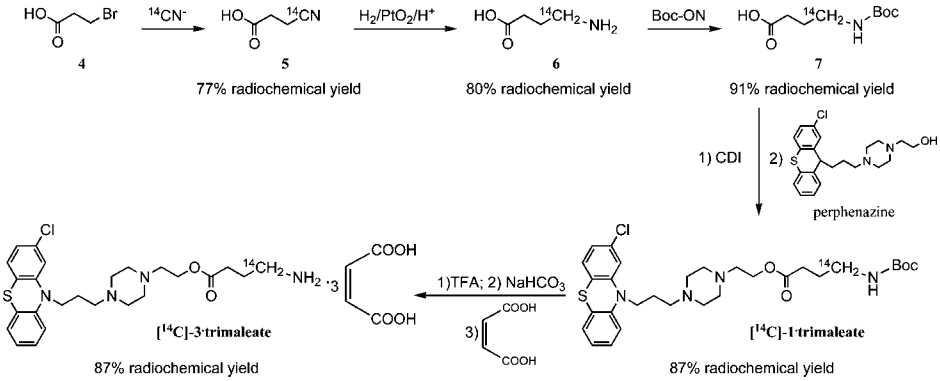
[a] Abbreviations: EPS, extrapyramidal symptoms; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; BBB, blood brain barrier; DA, dopamine; CDI, carbonyldiimidazole; DCC, dicyclohexylcarbodiimide; CI, chemical ionization; DMAP, 4-dimethylaminopyridine; TFA, trifluoroacetic acid.

10.1021/jm7012453 CCC: $40.75 © 2008 American Chemical Society
Published on Web 03/26/2008

Scheme 1. Synthesis of **3** and **4**



Scheme 2. Synthesis of Radiolabeled **3** Trimaleate



minor impurity stemming from a Michael addition product of the free base **3** and maleic acid. The final formulation of **3** for clinical studies was that of the trimesylate salt obtained by direct treatment of the Boc derivative **1** with methanesulfonic acid.

The synthesis of the radiolabeled **3** trimaleate salt prepared for pharmacokinetic studies is shown in Scheme 2.

To confirm the presence of intact **3** and its metabolites in the brain, the radiolabeled trimaleate salt was administered to rats. Brain slices obtained from different regions of the brain and whole brain homogenates were subjected to analysis for the presence of [^{14}C]-**3** and/or its metabolite [^{14}C]-GABA, qualitatively by autoradioluminography and for quantitatively at different time points by HPLC radiochromotography. The data showed that **3** transverses the BBB showing peak level of the labeled **3** after 5 min in the brain compartment, while total radioactivity was evenly distributed in the brain and reached a maximum level after 1 h. The complete radiological analysis is described and discussed elsewhere (Geffen et al., submitted for publication).

Biology. The typical antipsychotics perphenazine and fluphenazine and their respective GABA esters **3** and **4** were administered to rats, and their effects were compared to those of the parent neuroleptics for blood prolactin levels, EPS (catalepsy), and antipsychotic efficacy.

Prolactin Secretion. Most typical as well as some atypical neuroleptics such as risperidone induce hyperprolactinemia, and circulatory plasma prolactin levels serve as a sensitive biochemical marker for detecting agents that block central DA transmission.[25,26] In rats treated ip with perphenazine, fluphenazine, and their respective esters **3** and **4**, a maximum level of prolactin was detected after 1 h post-treatment, and although attenuated, the hormone concentration remained above basal level after 2 h, whereas in animals treated with 1% lactic acid (vehicle) the level was unchanged. These results indicate that **3** and **4** are as effective DA receptor antagonists as perphenazine and fluphenazine (Figure 1).

Catalepsy. The manifestation of EPS induced by typical neuroleptics was evaluated by the appearance of a stereotypic cataleptic behavior in rats following the neuroleptic treatment. Catalepsy was determined by the "wall descending test" (see the Experimental Section), which provides an assessment of animal muscle rigidity and lethargy and is an acceptable criterion

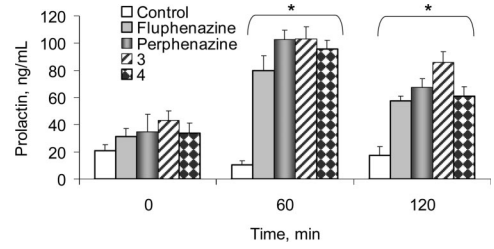


Figure 1. Effect of perphenazine and fluphenazine and their respective equimolar doses of GABA esters **3·3HCl** and **4·3HCl** on plasma prolactin levels. Rats (four/group) were treated ip with perphenazine (5 mg/kg), fluphenazine (7.5 mg/kg), or the equimolar doses of their respective esters **3·3HCl** and **4·3HCl.** Blood from animals, under light ether anesthesia, was drawn by puncturing their orbital vein prior to treatment and 1 and 2 h post-treatment. Prolactin levels were determined using a double-antibody Rat Prolactin RIA kit (Biocode Belgium). Compared to the control and the level of prolactin at time 0, the tested treatments significantly increased prolactin level (*t test $p < 0.05$).

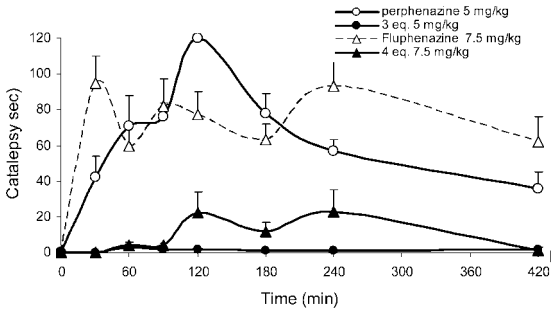


Figure 2. GABA esters **3·3HCl** and **4·3HCl** when administered ip to rats abolish or attenuate catalepsy. Wall descending test: Rats (six/group) were treated ip with perphenazine (5 mg/kg), fluphenazine (7.5 mg/kg) or equimolar doses of their respective esters **3·3HCl** and **4·3HCl**. Using the wall descending assay, the rats were monitored for catalepsy every 30 min and up to 420 min post treatment.

Table 1. Sedation Score in Rats Treated ip with Perphenazine (5 mg/kg) or Fluphenazine (7.5 mg/kg) and Their GABA Esters at Equimolar Concentrations[a]

	30 min	60 min	90 min	120 min	180 min	240 min
perphenazine	**	**	**	***	**	**
3·3HCl	0	0	*	*	*	*
fluphenazine	*	**	***	***	**	**
4·3HCl	*	**	**	**	*	*

[a] 0, active mobile animal; *, calm and mobile; **, calm and immobile; ***, ataxic and non-alert.

for EPS induced by antipsychotic drugs.[27] The time course of induced catalepsy was measured for perphenazine and fluphenazine and their equimolar doses of **3** and **4** dissolved in 1% lactic acid, administered ip. The results demonstrate that animals treated with perphenazine and fluphenazine exhibited typical cataleptic behavior detected already 30 min following treatment, that peaked at 2 h and was attenuated thereafter. The animals treated with **3** showed no catalepsy and those treated with **4** exhibited a very low cataleptic behavior detected only 2 h after treatment and was undetectable after 7 h (Figure 2).

At the time catalepsy was measured, animal sedation was evaluated in a double blind manner. The sedation score correlated with catalepsy, and at its peak, the animals treated with perphenazine and fluphenazine were ataxic and immobile for most of the time, while those treated with **3** were alert, calm, and mobile and those treated with **4** showed decreased mobility (Table 1). Subsequent experiments were conducted with **3** since it elicited lower cataleptic effects than **4.**

Effect of Orally Administered 3. To test the oral bioavailability and activity of **3**, the ester was administered by gavage to rats, and catalepsy by the "bar test" (see the Experimental Section) was evaluated as a function of dose and time. The obtained results showed evidence that **3** upon po administration was absorbed rapidly and induced effective DA receptor antagonism as reflected by an increase in prolactin levels in all treated animals (Figure 3).

Catalepsy upon Orally Administered 3. Since the prolactin assay indicated that **3** is orally bioavailable and increases prolactin levels, the effect of the ester on catalepsy was evaluated as a function of time. Perphenazine at 5 and 10 mg/kg elicited a dose-dependent catalepsy that peaked at 4–5 h post treatment, while **3** at equimolar doses imparted a significantly lower cataleptic behavior; moreover, the ester was well tolerated and caused decreased sedation compared to perphenazine (Figure 4A,B).

Based on the "bar test", the data showed a consistent and significant ($p < 0.05$) reduction in cataleptic behavior at all of the used concentrations of **3** compared to equimolar doses of

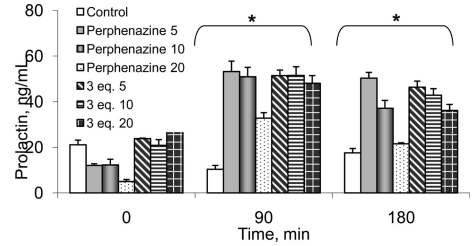


Figure 3. Plasma prolactin levels in rats treated po with perphenazine or **3·3HCl**. Rats divided into 5 groups (4/group) were treated po with 1% lactic acid (vehicle control), perphenazine 5 10 and 20 mg/kg, and the respective equimolar doses 7 and 14 and 28 mg/kg of **3·3HCl**. The prolactin level was determined prior-administration (time 0) and 90 and 180 min post-administration of the treatment. The prolactin determination was performed as described in Figure 1. Compared to the control and the level of prolactin at time 0, the tested treatments significantly (by *t* test) increased prolactin level (* $p < 0.05$).

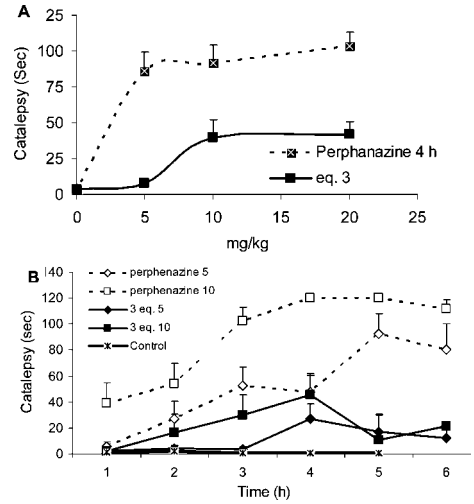


Figure 4. Catalepsy induced by orally administered **3·3HCl**. Bar test: Rats were treated po with perphenazine (5, 10, and 20 mg/kg) and equimolar doses of **3·3HCl** and catalepsy was determined 4 h after the treatment (A). The time course of catalepsy was determined in animals treated with 5 and 10 mg/kg perphenazine compared to the respective equimolar doses of **3·3HCl** (B).

perphenazine. Due to practical considerations, the maximal cataleptic signal measured was limited to 120 s. In reality, the absolute differences between perphenazine and **3** were greater. Moreover, muscular rigidity was apparent only in animals treated with perphenazine and not in those treated with the respective equimolar doses of **3**. Regarding the relation between time and effect of the drugs, the data demonstrate that maximum catalepsy was achieved for perphenazine and **3**, 4–5 h after treatment.

Neuroleptic Efficacy in a D-Amphetamine-Induced Hyperactivity and Motility Model. The rat psychosis model of D-amphetamine-induced hyperactivity is commonly used as an model for schizophrenia.[28] The hyperactivity induced by the D-amphetamine was manifested by increased wall-climbing attempts and head movements and was recorded double-blindly during a period of 2 min and 30 min after D-amphetamine administration. In this model, GABA administered as a single agent had no effect on reduction of the animal hyperactivity. Perphenazine abolished the hyperactivity; however, it was clear from observing the animals' posture that it induced sedation and catalepsy. In contrast, equimolar doses of the **3** reduced the wall-climbing attempts and head movements to the negative control levels without sedation and with minimal or no catalepsy (Figure 5).

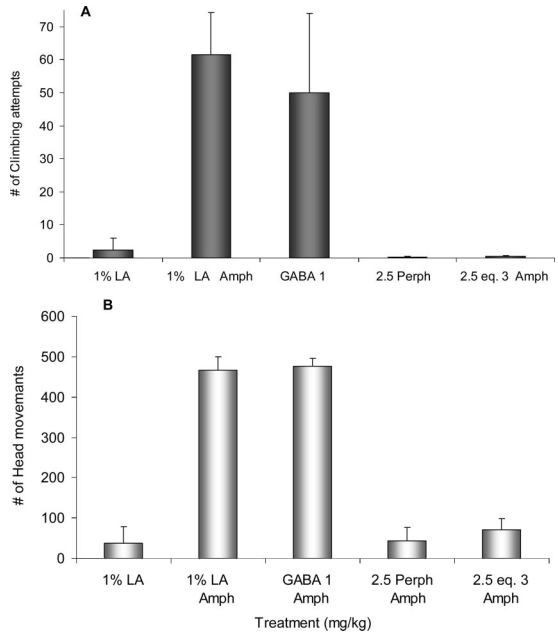


Figure 5. Perphenazine and **3·3HCl** abrogate hyperactivity induced by D-amphetamine in rats. Male Wistar rats divided into five groups (six/group) were treated po respectively with: vehicle (1% lactic acid, two groups); perphenazine (2.5 mg/kg); an equimolar dose of **3·3HCl**; and GABA (1 mg/kg). With the exception of one of the vehicle treated groups, which served as negative control, after 90 min all other animals received D-amphetamine (2.0 mg/kg, ip). The rats were then placed individually in barrels, and the number of head movements and climbing attempts on the barrel walls were recorded double-blindly.

Conclusion

In animal studies, **3** represents a new prototype of efficacious neuroleptics possessing GABAergic activity and reduced EPS and sedation. Compound **3**, as its trimesylate salt, was therefore chosen for advanced studies aimed at establishing its activity as a novel, orally active antipsychotic possessing decreased adverse side effects and improved therapeutic potential targeting positive and negative symptoms of schizophrenia. Further animal pharmacokinetic and toxicological studies will be published elsewhere (Gefen et al., submitted). A phase I clinical trial with **3** (**BL-1020**) on healthy volunteers indicated that the compound is safe, well tolerated, and has the potential of benefitting schizophrenic patients.[29] Phase II clinical trials are underway.

Experimental Section

Chemistry. General Procedures. ^{1}H and ^{13}C NMR spectra were obtained on 200, 300, and 600 MHz Bruker AC-200, AM-300, and DMX-600 spectrometers. Chemical shifts are expressed in ppm downfield from Me_4Si used as internal standard. The values are given on a δ scale. Mass spectra were obtained on a Varian Mat 731 spectrometer (CI = chemical ionization). HRMS were obtained on a VG AutoSpec E spectrometer. The reaction progress was monitored by TLC on silica gel (Merck, Art. 5554) or alumina. (Riedel-de Haen, Art. 37349). Flash chromatography was carried out on silica gel (Merck, Art. 9385). Commercially available compounds were used without further purification.

3-*tert*-Butoxycarbonylaminopropionic Acid 2-{4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl}ethyl Ester (1). Procedure A using carbonyldiimidazole (CDI): A mixture of *N*-*t*-Boc-GABA (1 equiv) and CDI (1.1 equiv) in DMF (5–10 mL) under N_2 was stirred for 1 h. Perphenazine (1 equiv) was added, and the mixture was stirred under N_2 at 90 °C for 24 h. The resulting slurry was evaporated and partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc (×2). The combined organic layer was washed with $NaHCO_3$ (×3) and brine (×2), dried ($MgSO_4$), filtered, and evaporated to give a residual yellowish oil. The crude product was chromatographed (silica gel, EtOAc/EtOH, 20:1) to give **1** as an oil (63% yield): ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, *t*-Bu), 1.82 (quint, J = 7.18 Hz, 2H, CH_2CH$_2$NHBoc), 1.90 (quint, J = 7.18 Hz, 2H, ArNCH$_2$CH_2), 2.35 (t, J = 8.97 Hz, 2H, CO$_2$CH_2), 2.42 (m, 10H, five NCH_2), 2.60 (t, J = 5.98 Hz, 2H, NCH_2CH$_2$O), 3.16 (q, J = 6.85 Hz, 2H, CH_2NHBoc), 3.84 (t, J = 7.2 Hz, 2H, ArNCH_2), 4.18 (t, J = 5.98 Hz, 2H, NCH$_2$CH_2O), 5.10 (bs, 1H, NH), 6.83 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 23.9 (*C*H$_2$CH$_2$NHBoc), 25.0 (ArNCH$_2$*C*H$_2$), 28.2 (*t*-Bu), 39.5 (*C*H$_2$CO$_2$), 45.1 (ArN*C*H$_2$), 52.9 (two N*C*H$_2$), 53.0 (two N*C*H$_2$), 55.2 (ArNCH$_2$CH$_2$*C*H$_2$), 56.3 (N*C*H$_2$CH$_2$O), 60.1 (*C*H$_2$NHBoc), 61.3 (NCH$_2$*C*H$_2$O), 78.8 (*C*Me$_3$), 115.6 (C$_1$, C$_{10}$), 122.0 (C$_3$), 122.7 (C$_8$), 123.2 (C$_5$), 124.5 (C$_6$), 127.2 (C$_7$, C$_4$), 127.6 (C$_9$), 132.9 (C$_2$), 144.2 (C$_{12}$), 146.2 (C$_{11}$), 155.8 (NCO$_2$), 172.9 (*C*O$_2$). Procedure B using dicyclohexylcarbodiimide (DCC): To an ice-cold solution of perphenazine (10 g, 24.8 mmol), *N*-*t*-Boc-GABA (6.04 g, 29.8 mmol), and 4-dimethyl aminopyridine (DMAP) (0.91 g, 7.4 mmol, 0.3 equiv) in CH_2Cl_2 (60 mL) was added DCC (6.44 g, 31.2 mmol) portionwise, and the mixture was stirred at room temperature for 16 h. The resulting slurry (precipitated dicyclohexyl urea, DCU) was ice-cooled for 2 h and filtered; the collected solid was washed with ice-cold CH_2Cl_2 (2 × 10 mL), the filtrate was evaporated, and the residue was redissolved in EtOAc (70 mL), cooled, and filtered again (to precipitate any remaining DCU). The EtOAc solution was then washed 2× with 5% citric acid solution (to remove any remaining DMAP), 2× with 1 M $NaHCO_3$ solution, and finally with brine (2 × 50 mL). The solution was then concentrated under vacuum at 40 °C to yield **1** as a viscous orange oil in 98% yield and a 99.5% area HPLC.

4-Aminobutyric Acid 2-{4-[3-(2-Chlorophenothiazin-10-yl)-propyl]piperazin-1-yl}ethyl Ester Trihydrochloride (3·Trihydrochloride). A solution of **1** and 4 N HCl in EtOAc was stirred for 2 h at room temperature. The solvent was evaporated, and the residue was further dried under high vacuum to give **3**, as a hygroscopic trihydrochloride salt, which was crystallized from a MeOH−ether mixture: ^{1}H NMR ($CDCl_3$) δ 1.93 (quint, J = 7.14 Hz, 2H, CH_2CH$_2$NH$_2$), 2.23 (m, 2H, ArNCH$_2$CH_2), 2.61 (t, J = 7.14 Hz, 2H, CO$_2$CH_2), 3.01 (m, 2H, CH_2NH$_2$), 3.33 (m, 2H, ArNCH$_2$CH$_2$CH_2), 3.48–3.87 (m, 10H, five NCH_2), 4.10 (t, J = 6.4 Hz, 2H, NCH$_2$CH_2O), 4.48 (m, 2H, ArNCH_2), 7–7.31 (m, 7H, Ar); ^{13}C NMR ($CDCl_3$) δ 22.3 (*C*H$_2$CH$_2$NH$_2$), 22.9 (ArNCH$_2$*C*H$_2$), 31.1 (*C*H$_2$CO$_2$), 39.6 (CH$_2$NH$_2$), 44.8 (ArN*C*H$_2$), 49.4 (two N*C*H$_2$), 49.6 (two N*C*H$_2$), 55.3 (ArCH$_2$CH$_2$*C*H$_2$), 56.1 (NCH$_2$*C*H$_2$O), 58.6 (N*C*H$_2$CH$_2$O), 116.7 (C$_{10}$), 117.2 (C$_1$), 123.5 (C$_3$), 124.2 (C$_8$), 125.4 (C$_5$), 126.4 (C$_6$), 128.2 (C$_7$), 128.6 (C$_9$), 128.8 (C$_4$), 134.2 (C$_2$), 145.0 (C$_{12}$), 147.4 (C$_{11}$), 173.0 (*C*O$_2$); MS (CI/CH$_4$) *m*/*z* 403.09 (MH$^+$ − C$_4$H$_7$NO, 100), 489.18 (MH$^+$, 1.7).

4-[3-(2-Chloro-10*H*-phenothiazine-10-yl)propyl]-1-piperazine-ethyl 4-Aminobutyryl Ester Tritrifluoroacetate (3·3TFA) and Trimaleate (3·3HOOCCH=CHCOOH) Salts. Compound **1** (100 g, 0.17 mol) was dissolved in CH_2Cl_2 (400 mL) under N_2 and cooled to 10 °C. Trifluoroacetic acid (TFA) (100 mL) was added dropwise while the temperature was maintained below 20 °C. When the addition was complete, the solution was heated to 35 °C and maintained at this temperature until reaction completion (16 h). The solution was then evaporated, and the residual oil containing **3·3TFA**, used without further purification, was redissolved in CH_2Cl_2, cooled to 0 °C, and added to a 1 M $NaHCO_3$ solution (1.3 L). The layers were separated, and to the lower CH_2Cl_2 phase was added maleic acid (39.4 g, 0.34 mmol) dissolved in 2-propanol (200 mL). The obtained slurry was cooled, stirred for 1 h at room temperature, and filtered, and the precipitant was washed with 2-propanol (2 × 100 mL), slurried in purified water, washed with additional purified water, and lyophilized to give **3·trimaleate** (98% yield, 99.3% HPLC area from silica-based reversed-phase column, eluted with a 0.1% formic acid/acetonitrile gradient, and monitored at 254 nm): mp 161.9 °C by differential scanning calorimetry: ^{1}H NMR ($CDCl_3$) δ 1.84 (q, 2H, J = 7.3 Hz, 2H, CH_2CH$_2$NH$_2$), 2.07 (m, 2H, ArNCH$_2$CH_2), 2.39 (t, J = 7.1 Hz, 2H, CO$_2$CH_2), 2.72 (m, 2H, CH_2C=O + NCH$_2$CH_2O), 2.89 (t, J = 4.9 Hz, 2H, ArNCH$_2$CH$_2$CH_2), 3.04 (bm, 8H NCH_2CH_2N + 2H NCH_2+ 2H CH$_2$NH$_2$), 3.97 (t, J = 6.4 Hz, 2H, CH_2O), 4.15 (t, J = 6.12 Hz, 2H, ArNCH_2), 6.18 (s, 6H, C*H*=C*H*), 6.87–7.16 (m, 7H,

Ar); ^{13}C NMR ($CDCl_3$) δ 21.7 ($CH_2CH_2NH_2$), 22.4 ($ArNCH_2CH_2$), 30.3 (CH_2CO_2), 38.2 (CH_2NH_2), 44.0 ($ArNCH_2$), 49.8 (two NCH_2), 51.0 (two NCH_2), 53.5 ($ArCH_2CH_2CH_2$), 54.9 (NCH_2CH_2O), 60.7 (NCH_2CH_2O), 115.9 (C_{10}), 115.9 (C_1), 116.4 ($CH{=}CH$), 122.5 (C_3), 123.1 (C_8), 123.3 (C_5), 123.8 (C_6), 127.4 (C_7), 127.9 (C_9), 128.3 (C_4), 132.6 (C_2), 143.8 (C_{12}), 146.2 (C_{11}), 167.1 (CH-CO_2), 172.1 (CO_2).

Biology. Animals. Wistar male rats (150–230 g) (Harlan, Israel) were divided two to three per cage and housed under controlled conditions for one week prior to the experiments.

Prolactin secretion. Rats (four/group) were treated ip or po with the indicated drugs. Blood was collected from the animals under light ether anesthesia at the indicated time points by puncturing their orbital vein. Samples in EDTA were placed in ice. The plasma, separated by centrifugation at 1600*g* for 20 min, was frozen at −78 °C. Plasma prolactin levels were determined using a double antibody rat prolactin radioimmunoassay kit (Biocode, Liege, Belgium) according to the manufacturer's protocol.

Catalepsy. The induced EPS were manifested by the induction of a stereotypic cataleptic behavior in rats.[27,28] The methods used were as follows. (a) Bar test: Rats were placed on a flat surface with their anterior limbs leaning on a flat bar (5.5 cm height). Catalepsy was determined by the time it took an animal to descend and reach the flat surface. (b) Wall descending test: Catalepsy was determined by the time it took an animal hanging on a cage wall to move its hind legs and reach the cage floor. The measurements were performed hourly, and the animals were tested individually at each time point and followed for a maximal period of 120 s.

Behavioral Observations. The animals' sedation score was carefully monitored during experimentation, and the status of their alertness was double-blindly recorded.

D-Amphetamine-Induced Hyperactivity. The hyperactivity model was based on a reported procedure[28] and modified as follows. Male Wistar rats divided into five groups (six/group) were treated po respectively with vehicle (1% lactic acid, two groups), perphenazine (2.5 mg/kg), an equimolar dose of **3**, and GABA (1 mg/kg). With the exception of one of the vehicle treated groups, which served as negative control, after 90 min all other animals received D-amphetamine (2.0 mg/kg, ip). The rats were than placed individually in barrels, and their motility was assessed after 30 min. The hyperactivity of each animal in the course of 120 s, manifested by the increased number of head movements (HM) and increase in climbing attempts on the barrel walls, was recorded double-blindly.

Acknowledgment. The generous support for this work by BiolineRX, Jerusalem, Dr. Shlomit Halachmi, and the "Marcus Center for Pharmaceutical and Medicinal Chemistry" at Bar Ilan University is gratefully acknowledged.

Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM7012453