Pharmacological Profile of 2-Bromoterguride at Human Dopamine D₂, Porcine Serotonin 5-Hydroxytryptamine 2A, and α₂C-Adrenergic Receptors, and Its Antipsychotic-Like Effects in Rats


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Received April 25, 2013; accepted July 16, 2013

ABSTRACT

Dopaminergic, serotonergic, and adrenergic receptors are targets for therapeutic actions in schizophrenia. Dopamine D₂ receptor partial agonists such as aripiprazole represent a treatment option for patients with this severe disorder. The ineffectiveness of terguride, another D₂ receptor partial agonist, in treating schizophrenia was recently attributed to its considerably high intrinsic activity at D₂ receptors. In this study, we used functional assays for recombinant D₂ receptors and native 5-hydroxytryptamine 2A (5-HT₂A), α₂C-adrenergic, and histamine H₁ receptors to compare the pharmacological properties of terguride and three of its halogenated derivatives (2-chloro-, 2-bromo-, 2-iodoterguride) with those of aripiprazole. Subsequently, we studied the antidopaminergic effects of 2-bromoterguride using amphetamine-induced locomotion (AIL). Its influence on spontaneous behavior was tested in the open field. Extrapyramidal side effect (EPS) liability was evaluated by catalepsy test. In a guanosine 5′-O-(3-[35S]thio)triphosphate ([35S]GTPγS) binding assay, 2-chloro-, 2-bromo-, and 2-iodoterguride produced intrinsic activities at human D₂ short (hD₂s) receptors that were half as high as the intrinsic activity for terguride; aripiprazole lacked agonist activity. 2-Bromoterguride and aripiprazole activated D₂s receptor-mediated inhibition of cAMP accumulation to the same extent; intrinsic activity was half as high as that of terguride. All compounds tested behaved as antagonists at human D₂ long (hD₂l)/Gα₁ receptors. Compared with aripiprazole, terguride and its derivatives displayed higher affinity at porcine 5-HT₂A receptors and α₂C-adrenoceptors and lower affinity at H₁ receptors. 2-Bromoterguride inhibited AIL and did not induce catalepsy in rats. Because of its in vitro and in vivo properties, 2-bromoterguride may be a strong candidate for the treatment of schizophrenia with a lower risk to induce EPS.

Introduction

Standard, or typical, antipsychotic drugs such as haloperidol display prominent antagonist potency at dopamine D₂ receptors, with substantial risk of extrapyramidal side effects (EPS) (Baldessarini and Tarazi, 2006). In contrast, atypical antipsychotics, drugs of which clozapine is the prototype, produce fewer EPS than does haloperidol (Leucht et al., 2009). Clozapine, olanzapine, and risperidone have been shown to be more efficacious than typical antipsychotic drugs in the treatment of positive symptoms (affective flattening, alogia, avolition, anhedonia) (Leucht et al., 2009). However, clozapine can induce life-threatening hematologic side effects (anagranulocytosis, leukopenia), and clozapine, olanzapine, and risperidone cause considerable weight gain (Kroeze et al., 2003; Roth et al., 2004). Atypical antipsychotics have different pharmacologic profiles at D₂ receptors, 5-hydroxytryptamine (serotonin) (5-HT) (5-HT₁A, 5-HT₂A, 5-HT₂C, 5-HT₆, 5-HT₇), α₁A, α₁B, α₂C), and histamine H₁ receptors (Kroeze et al., 2003; Roth et al., 2004; Meltzer et al., 2012).

The development of aripiprazole, a D₂ receptor partial agonist, represents a treatment option for patients with schizophrenia (Burris et al., 2002; Shapiro et al., 2003). As expected for an atypical antipsychotic agent, aripiprazole also...
exhibited high affinity for multiple serotonin receptors and α-adrenoceptors (Kroeze et al., 2003). Consequently, it was hypothesized that “the balance of partial agonism and antagonism at a multiplicity of receptors is responsible for its efficacy in schizophrenia and related disorders” (Roth et al., 2004). Terguride, [1,1-diethyl-3-(6-methyl-8α-ergolinyl)urea] fulfills the criteria of a nonelective drug that interacts with a multiplicity of receptors playing a role in the pathophysiology and treatment of schizophrenia (Roth et al., 2004). Indeed, terguride acts as a D2 receptor partial agonist of appreciable intrinsic activity and as an antagonist at a number of serotonin receptor and α1- and α2-adrenoceptor subtypes (Newman-Tancredi et al., 2002a,b). Terguride was tested in schizophrenia but was found to be clinically efficacious only in reducing negative symptoms (Olbrich and Schanz, 1988, 1991). The relatively high intrinsic activity for terguride at D2S receptors, which was twice as high as that of aripiprazole (Tadori et al., 2005), has recently been suggested to be responsible for the insufficient clinical efficacy of terguride (Natesan et al., 2011). Accordingly, it seems likely that a potential antipsychotic drug should exert high affinity but low intrinsic activity at D2 receptors (Natesan et al., 2011) and, consistent with the pharmacological properties of atypical antipsychotic drugs, affinity for other biogenic amine receptors that play a role in schizophrenia.

There is still a need for better antipsychotics with improved efficacy and reduced adverse effects to treat the various symptoms of schizophrenia. Against this background, we studied the pharmacology of 2-chloroterguride, 2-bromoterguride, and 2-iodoterguride in comparison with terguride and aripiprazole using 1) competition binding assays for recombinant human D2S (hD2S) and D2L (hD2L) receptors, stably expressed in Chinese hamster ovary (CHO) cells, to estimate drug affinities; 2) guanosine 5′-O-[(αβ)32P]triphosphate ([32P]GTP[S]) binding assays for hD2S and hD3L receptors to measure G protein activation; 3) hD2S receptor-mediated inhibition of forskolin-stimulated cyclic AMP accumulation; 4) porcine coronary arteries to measure 5-HT-induced contraction mediated by 5-HT2 receptors; 5) porcine pulmonary arteries to measure 5-HT2A receptors; and 6) porcine pulmonary veins to measure histamine-induced contraction via activation of histamine H1 receptors. In addition, we examined 7) the effect of 2-bromoterguride on spontaneous behavior, 8) its antidepressive efficacy using amphetamine-induced locomotion (AIL), and 9) its EPS liability using the catalepsy test in rats. We selected 2-bromoterguride for our in vivo studies because this drug is the dihydro derivative of bromerguride (2-bromolisuride), a drug that has shown atypical antipsychotic properties (Löschmann et al., 1992).

Materials and Methods

Animals. For in vitro organ-bath studies, pig hearts and lungs were used, which were obtained from the Lehr- und Versuchsanstalt für Tierzucht und Tierhaltung (Teltow-Ruhlsdorf, Germany). For in vivo studies, a total of 84 male Sprague-Dawley rats (Élevage Janvier, Le Genest Saint Isaac, France) aged 10–11 weeks were used. Animals were housed in Makrolon cages (type IV; n = 3–4) under standard conditions (room temperature: 22 ± 2°C, relative humidity: 55 ± 10%) on a 12-hour light/dark schedule (lights on at 6:00 AM). They received free access to standard laboratory chow (sniff, Soest, Germany) and tap water. All experiments were performed in accordance with the guidelines of the German Animal Protection Law and were approved by the Berlin State Authority (“Landesamt für Gesundheit und Soziales”).

Drugs. UK-14304 was a gift from Allergan Pharmaceuticals (Westport, County Mayo, Ireland). The following drugs were purchased: di-amphetamine from Berlin-Chemie (Berlin, Germany), aripiprazole from Toronto Research Chemicals (Toronto, ON, Canada), cocaine hydrochloride and histamine dihydrochloride from Merck (Darmstadt, Germany), and 5-hydroxytryptamine creatinine sulfate from Agros Organics (Geel, Belgium). 4-(3-Butoxy-4-methoxybenzyl)imidazolin-2-one, Cremophor EL, 3-isobutyl-1-methylxanthine (IBMX), indomethacin, αN-nitro-l-arginine methyl ester (L-NAME), norepinephrine bitartrate, prazosin hydrochloride, propranolol hydrochloride, and quinpirol hydrochloride were obtained from Sigma-Aldrich (Taufkirchen, Germany). Terguride, 2-chloroterguride, 2-bromoterguride, and 2-iodoterguride were obtained from Alfarma (Cernosice, Czech Republic). (S)-(-)-1-D,4-Dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-3-pyrindinecarboxylic acid methyl ester ([S](-)-Bay K 8644) was purchased from Tocris Biosciences (Bristol, UK), and ketanserin tartrate and haloperidol were obtained from Janssen Pharmaceuticals (Beerse, Belgium).

For the in vitro experiments, the drugs were dissolved in distilled water, dimethyl sulfoxide (e.g., ropinirole; aripiprazole in assays using recombinant receptors), 50% (v/v) ethanol (indomethacin and prazosin), or a mixture of 50% (v/v) ethanol and an equimolar amount of 1 N HCl (aripiprazole in assays using native receptors) to a final stock solution. Stock solutions were stored at −18°C and were freshly diluted in distilled water, phosphate-buffered saline (PBS), or HEPES buffer before the beginning of the experiment. The final concentrations of ethanol and dimethyl sulfoxide did not exceed 0.1 and 0.01%, respectively.

For the in vivo experiments, aripiprazole and 2-bromoterguride were made soluble in 15% Cremophor EL, and haloperidol and di-amphetamine were dissolved in 0.9% saline. The appropriate dose of 2-bromoterguride was determined in pilot experiments.

Groups of three rats were treated with 0.1, 0.3, and 1.0 mg/kg 2-bromoterguride to analyze the influence on spontaneous and cataleptic behavior. As 1.0 mg/kg 2-bromoterguride produced a profound sedative effect on spontaneous locomotion, all subsequent experiments were performed with 0.1 and 0.3 mg/kg 2-bromoterguride. Drugs were freshly prepared before being injected intraperitoneally (injection volume: 1.0 ml/kg body weight).

Radioligand Binding Studies on hD2S and hD2L Receptors. Competition binding experiments were performed as described previously elsewhere (Hüner et al., 2000) using membrane preparations from CHO cells stably expressing either hD2S or hD2L receptors (Hayes et al., 1992). Assays were run with membranes at protein concentration per well of 2 μg/ml for D2S and 4 μg/ml for D2L and [3H]Flupentixol (specific activity 84 Ci/mmol; PerkinElmer, Rodgau, Germany) at final concentrations of 0.1 and 0.2 nM for D2S and D2L receptors, respectively. The Kd values for D2S and D2L receptors were 0.040 and 0.12 nM; the Bmax values were 3470 fmol/mg and 1310 fmol/mg, respectively. Membranes, radioligand, and test compound were incubated for 60 minutes at 37°C in binding buffer (50 mM Tris-HCl, 1 mM EDTA, 5 mM MgCl2, 100 μg/ml bacitracin, and 5 μg/ml soybean trypsin inhibitor at pH 7.4). Incubations were terminated by rapid filtration through Whatman GF/C filters presoaked with 0.3% polyethyleneimine. The filters were rinsed 5 times with ice-cold Tris-NaCl buffer (50 mM Tris-HCl, 120 mM NaCl at pH 7.4). After 3 hours of drying at 60°C, the filters were counted for radioactivity using a scintillation spectrometer. Nonspecific binding was determined in the presence of 10 μM haloperidol. Specific binding was about 90% of the total binding. Protein concentration was determined by the method of Lowry using bovine serum albumin as a standard.

GTPyS Binding Studies on hD2S Receptors. The agonist potencies of the compounds were investigated in a [35S]GTPyS assay using membranes of CHO cells stably expressing hD2S receptors.
(Görnemann et al., 2008). Homogenates of membranes (B_{max} = 3300–4800 fmol/mg) were diluted in HEPES buffer (20 mM HEPES, 10 mM MgCl₂, 100 mM NaCl, 40 μM/ml saponin; pH 7.4) and incubated at 37°C with 1 μM GDP (HEPES buffer), the reference agonist quinpirole, and the test compound (ergot derivative) applying 10 different concentrations (0.01–1000 nM) as triplicates at a final volume of 200 μl in 96-well microplates.

In separate experiments, quinpirole (0.1 nM to 1 mM), aripiprazole (1 μM to 10 μM), and quinpirole (5 μM) plus aripiprazole (1 μM to 10 μM) were used. After 30 minutes, 0.1 nM [³⁵S]GTPγS (specific activity 1250 Ci/mmol; PerkinElmer Life and Analytical Sciences, Waltham, MA) were added, and the incubation was continued for a further 30 minutes. The experiment was terminated by rapid filtration through GF/B filters using an automated cell harvester; the filters were washed five times with ice-cold washing buffer (140 mM NaCl, 10 mM KCl, 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄, pH 7.4), dried at 60°C for 3 hours, and the trapped radioactivity was counted in a microplate scintillation counter. [³⁵S]GTPγS binding data from each individual experiment were expressed as a percentage of the full response to quinpirole.

**GTPγS Binding Studies on hD₂/Goα Receptors**. Membrane preparations of transiently transfected human embryonic kidney 293 (HEK293) cells that expressed hD₂ receptors coupled to Goα, were used in a [³⁵S]GTPγS assay, as recently described elsewhere (Tschammer et al., 2011). Receptor expression determined in saturation experiments was 1640–1850 fmol/mg protein. The assay was performed in 96-well plates at a final volume of 200 μl of incubation buffer (20 mM HEPES, 10 mM MgCl₂, 100 mM NaCl, and 70 μg/ml saponin; pH 7.4). Membranes (30 μg/ml membrane protein), quinpirole (0.1 nM to 1 mM), the test compound (0.01 nM to 100 μM), quinpirole (1 μM) plus the test compound (0.1 mM to 100 μM), and 10 μM GDP were preincubated in the absence of [³⁵S]GTPγS for 30 minutes at 37°C. In additional experiments, quinpirole (0.1 nM to 1 mM), aripiprazole (1 μM to 10 μM), and quinpirole (5 μM) plus aripiprazole (1 μM to 10 μM) were used.

After the addition of 0.10 nM [³⁵S]GTPγS, membranes were incubated for an additional 30 minutes at 37°C. Incubation was terminated by filtration through Whatman GF/B filters soaked with ice-cold PBS. The filter-bound radioactivity was measured as described previously. Three to eight experiments per compound were performed with each concentration in triplicate. [³⁵S]GTPγS binding data from each individual experiment were expressed as a percentage of the full response to quinpirole.

**Inhibition of cAMP Accumulation**. Inhibition of forskolin-stimulated cAMP accumulation mediated via hD₂ receptors was measured using the bioluminescence-based CAMP-Glo assay (Promega, Madison, WD) according to the manufacturer’s instructions. In brief, CHO cells stably expressing hD₂ receptors were seeded into a half-area 96-well plate (5000 cells/well) 24 hours before the experiment. Cells were washed with PBS (pH 7.4) to remove traces of serum and then incubated with the test compound (10 μM to 100 μM) dissolved in serum-free medium containing forskolin (20 μM), 3-isobutyl-1-methylxanthine (IBMX; 500 μM), and 4-(3-butoxy-4-methoxybenzyl)imidazol-2-one (Ro 20-1724; 100 μM). After 15 minutes of incubation at 25°C, cells were lysed with CAMP-Glo lysis buffer (for 15 minutes), followed by the kinase reaction performed by the addition of CAMP-Glo reaction buffer containing protein kinase A (20 minutes). The final step was the addition of an equal volume of kinase-Glo reagent and the measurement of bioluminescence on a Victor3V microplate reader (PerkinElmer Life and Analytical Sciences).

Four to seven experiments per test compound were performed with each concentration in triplicate. The effects on cAMP accumulation were expressed as a percentage of the full response to quinpirole.

**Tissue Bath Studies**. Pig hearts and lungs were placed in ice-cold oxygenated Krebs-Henseleit solution (KHS; 95.5% O₂/CO₂) of the following composition: 118 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl₂ (2.5 mM for pulmonary arteries and veins), 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 10 mM p-glucose (pH 7.4). Coronary arteries (left anterior descending and left circumflex) were removed from the hearts, and small branches of pulmonary arteries and veins were dissected from the lungs. The tissues were cleaned of fat and adhering tissue. The pulmonary arteries and veins were stored overnight at 4°C in previously gassed KHS containing indomethacin (30 μM). Preliminary experiments have shown that tissue storing overnight does not impair the contractility of the smooth muscle.

On the next day, the vessels were cut into rings (3–4 mm long and 2–3 mm inner diameter). Vascular rings were horizontally suspended between two L-shaped stainless steel hooks (300 μm diameter). The tissues were mounted in water-jacketed 20-ml organ chambers and constantly exposed to oxygenated KHS (pH 7.4, 37°C). Preparations were connected to an isometric force transducer (FMI TIM-1020; FMI Föhr Medical Instruments, Seeheim-Jugenheim, Germany) attached to a TSE 4711 transducer coupler (TSE Systems, Bad Homburg, Germany) and a Siemens C 1016 compensograph (Siemens AG, Erlangen, Germany) for the continuous recording of changes in tension.

**Porcine Coronary Arteries (Functional 5-HT₂A Receptor Assay)**. Resting tension was adjusted to 20 nN at the beginning of the experiment. The tissues were stabilized for 60 minutes with replacement of the bathing medium after 30 minutes. During the ensuing equilibration period (160 minutes), the vessels were stimulated twice with KCl (50 mM). The rings were rinsed with KHS for 5 minutes to wash out KCl. l-NAME (100 μM) was added to each tissue bath to inhibit endothelial nitric-oxide synthase. After an additional 30 minutes, the rings were stimulated with 5-HT (1 μM). The rings were rinsed with KHS for 10 minutes to wash out the 5-HT.

A single cumulative concentration-response curve (CRC) to 5-HT was constructed in the absence or presence of antagonist. Antagonists were added to the bathing medium 60 minutes before the construction of the 5-HT curve. Contractile effects were expressed as a percentage of the 5-HT–induced precontraction. All experiments were performed in the continuous presence of prazosin (0.1 μM), cocaine (10 μM), and indomethacin (5 μM) to block α₁-adrenoceptors and to inhibit neuronal uptake of 5-HT and vascular eicosanoid production by cyclooxygenase, respectively.

**Porcine Pulmonary Arteries (Functional α2B-Adrenoceptor Assay)**. Arterial rings were stabilized for 90 minutes with bath fluid replacements every 30 minutes. During a subsequent equilibration period of 180 minutes, vessels were stimulated 4 times with 1 μM norepinephrine (NE) with 6 minutes washings after each contractile challenge. This procedure was considered to yield stable and reproducible contractions. The tension was repeatedly readjusted to 20 mN and remained unchanged after the fourth NE (1 μM) stimulation. A single CRC to UK-14304 was constructed on each arterial ring described previously elsewhere (Jantschak and Pertz, 2012). Antagonists were added 2 hours before the construction of the CRC to UK-14304.

Contractile effects were expressed as a percentage of the fourth NE-induced contraction. Cocaine (10 μM), prazosin (0.03 μM), and propranolol (1 μM) were continuously present in the bath fluid to block neuronal uptake of NE and α₁- and β-adrenoceptors.

**Porcine Pulmonary Veins (Functional Histamine H₁ Receptor Assay)**. Resting tension was adjusted to 10 nN at the beginning of the experiment. The tissues were stabilized for 60 minutes with replacement of the bathing medium after 30 minutes. During the ensuing equilibration period (100 minutes), the vessels were stimulated 3 times with histamine (3 μM). The rings were rinsed with KHS for 8 minutes to wash out the histamine. A single cumulative CRC to histamine was constructed on each venous ring in the absence or presence of antagonist. Antagonists were added 30 minutes before the construction of the histamine curve.

Contractile effects were expressed as a percentage of the third histamine-induced contraction. Cocaine (10 μM) and propranolol (1 μM) were continuously present in the bath fluid to block neuronal histamine uptake and β-adrenoceptors.
In Vivo Experimental Procedure. Rats were handled daily for 1 week before testing. All experiments were conducted during the light phase between 8:00 AM and 1:00 PM in a noise-proof chamber with a light intensity of 100 lux in the center of the floor. Test naive animals were used to examine drug-induced effects on spontaneous behavior ($n = 30$) and AIL ($n = 48$), respectively. For the catalepsy test, 38 of these rats were tested a second time at random with at least 1 week in between to allow clearance of the drugs.

Catalepsy. Rats were tested at two different designs: placing both forepaws on a 9 cm block (block test) or placing the rat on an inclined grid (60°; grid test). Latency to voluntarily remove one paw from the block or a directed movement on the grid was considered to be the end of the test (descent latency). The cutoff time for both tests was set to 150 seconds. The animals were first examined 3 times on the block immediately followed by three test sessions on the inclined grid. Each test was performed at 15, 30, 60, 90, 120, and 150 minutes after the injection of 2-bromoterguride (0.1 and 0.3 mg/kg), haloperidol (0.5 mg/kg), or vehicle (15% Cremophor EL). The average descent latency was recorded for each rat at each time point. Catalepsy was scored from 0 to 5, according to the estimated time (in minutes): 0 $\leq$ 0.00–0.08; 1 $\leq$ 0.09–0.35; 2 $\leq$ 0.36–0.80; 3 $\leq$ 0.81–1.42; 4 $\leq$ 1.43–2.24; 5 $\leq$ 2.25 (Ahlenius and Hillegaart, 1986). An animal was considered cataleptic with a score $\geq 2$ (Wadenberg et al., 2001).

AIL and Spontaneous Behavior in the Open Field. The effect of 2-bromoterguride on AIL was evaluated in two open-field boxes ($50 \times 50 \times 32$ cm; gray PVC; in-house production). The distance traveled (in centimeters) was recorded and analyzed by a computer-based system (VideoMot2; TSE Systems, Bad Homburg, Germany). Animals were administered with either 2-bromoterguride (0.1 and 0.3 mg/kg), aripiprazole (3.0 mg/kg), or vehicle (15% Cremophor EL) and were placed into the open field for a 30-minute habituation period. Subsequently, amphetamine (1.5 mg/kg) or vehicle (0.9% saline) was injected, and the animals were immediately returned to the open field for a 120-minute recording period.

For testing spontaneous behavior of 2-bromoterguride in the open field, the drug was injected, and then the rat was placed back into the home cage. After 30 minutes, the rats were placed in the open-field arenas, and their behavior (locomotor activity [centimeters], rearings [counts]) was measured for 30 minutes.

Data Presentation and Analysis. Data are presented as mean values $\pm$ S.E.M. for $n$ individual experiments or $n$ animals. Data were analyzed and presented using the nonlinear curve-fitting program GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA) or SigmaPlot 11 (Systat Software, Erkrath, Germany), which allowed estimation of IC$_{50}$, $K_i$ (according to the Cheng-Prusoff equation), EC$_{50}$, and $E_{\text{max}}$ values (percentage of the maximal response to a reference compound). $K_i$ and EC$_{50}$ are presented as p$K_i$ (negative logarithm of $K_i$) and pEC$_{50}$ (negative logarithm of EC$_{50}$), respectively. Antagonist affinities (p$K_B$ = $-\log K_B$) for inhibition of quinpirole-stimulated $[^{35}\text{S}]$GTP$\gamma$S binding were calculated according to Lazareno and Birdsall (1993): $K_B = \text{IC}_{50}/[2 + ([A]/\text{EC}_{50})^{n_H}1/\alpha_H} - 1$, where IC$_{50}$ is the inhibitory concentration$_{50}$ of the antagonist, $[A]$ is the fixed

### Table 1

<table>
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<th>Ligand</th>
<th>$D_{2S}$</th>
<th>$D_{2L}$</th>
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<td>2-Iodoterguride</td>
<td>9.70 $\pm$ 0.12</td>
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Fig. 1. Effects of terguride, 2-halogenated derivatives of terguride, and aripiprazole at hD$_{2S}$ receptors stably expressed in CHO cells. (A) Partial agonist effects of terguride and its 2-halogenated derivatives (inset, full agonist effect of quinpirole). (B) Effects of aripiprazole alone or versus quinpirole (5 μM). $[^{35}\text{S}]$GTP$\gamma$S binding is expressed as a percentage relative to the effect of the full agonist quinpirole. Points are mean $\pm$ S.E.M. of four to eight individual experiments.
Dopamine hD2S and hD2L Receptor Binding. Terguride and its 2-halogenated derivatives showed subnanomolar affinities at hD2S and hD2L receptors stably expressed in CHO cells (Table 1). Affinities of 2-chloroterguride, 2-bromoterguride, and 2-iodoterguride were not different, neither at D2S nor at D2L receptors (P > 0.05). 2-Chlorterguride and 2-bromoterguride had a slight but significant preference for D2S over D2L receptors (P < 0.05).

**Results**

**Effects on GTPγS Binding at hD2S Receptors Stably Expressed in CHO Cells.** Terguride and its 2-halogenated derivatives behaved as low-efficacy partial agonists compared to the full agonist quinpirole as measured by hD2S receptor-mediated incorporation of [35S]GTPγS (Fig. 1A; Table 2). Terguride showed the highest intrinsic activity, which was 2- to 3-fold higher than the efficacies of 2-chloroterguride, 2-bromoterguride, or 2-iodoterguride. Aripiprazole showed no agonist activity but behaved as an antagonist of quinpirole-induced [35S]GTPγS incorporation (Fig. 1B). The antagonist potency for aripiprazole (pK([B] 5.70 ± 0.18; n = 7) was in the same range as the affinity (pK([B] 9.23) reported by Lawler et al. (1999).

**Effects on cAMP Accumulation at hD2S Receptors Stably Expressed in CHO Cells.** Selected compounds (quinpirole, terguride, 2-bromoterguride, and aripiprazole) were tested for their ability to inhibit cAMP production. Terguride acted as a partial agonist with appreciable intrinsic activity (0.57 versus 1.0 for quinpirole). In contrast, intrinsic activity for 2-bromoterguride was half as high (0.28) as that for terguride. Intrinsic activity for 2-bromoterguride and aripiprazole was the same (Fig. 2; Table 2).

**TABLE 2**

Pharmacological properties of terguride, 2-halogenated derivatives of terguride, and aripiprazole at hD2S receptors (stably expressed in CHO cells) and hD2S/Gs, receptors (stably expressed in HEK293 cells) determined in a [35S]GTPγS and a cAMP accumulation assay. Data are mean ± S.E.M. of four to eight individual experiments. E_{max} is expressed as percentage of the maximal response to the full agonist quinpirole. The pK([B] for inhibition of quinpirole-stimulated [35S]GTPγS binding was estimated according to Lazareno and Birdsall (1993).

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<th>Compound</th>
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<th>D_{2S/Gs}</th>
<th>pK([B])</th>
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<th>E_{max}</th>
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<tr>
<td>Aripiprazole</td>
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<td>–</td>
<td>9.32 ± 0.23</td>
<td>7.06 ± 0.22</td>
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</table>

ND, not determined.
Effects on GTPγS Binding at hD2L/Gαo Receptors Stably Expressed in HEK293 Cells. Dopamine D2L receptors are coupled to their effectors by G<sub>i</sub> and G<sub>o</sub> proteins (Cordeaux et al., 2001). We measured hD2L receptor-mediated incorporation of [35S]GTPγS in the presence of Gαo. Using this system, terguride and its 2-halogenated derivatives were devoid of agonist activity but behaved as antagonists of quinpirole-induced [35S]GTPγS incorporation (Fig. 3; Table 2). Antagonist potencies (p<sub>K<sub>B</sub></p> estimated in these experiments were in good agreement with affinities (p<sub>K<sub>i</sub></p> from our binding studies. Aripiprazole had the same pharmacological properties as the ergot derivatives, with no agonist activity but inhibition of quinpirole-induced [35S]GTPγS incorporation (Fig. 3E). Antagonist potency (p<sub>K<sub>B</sub></p> 9.32 ± 0.23; n = 6, see Table 2) for aripiprazole was in line with the affinity (p<sub>K<sub>i</sub></p> 9.28) reported in the literature (Lawler et al., 1999).

Effects at Smooth Muscle 5-HT<sub>2A</sub> Receptors in Porcine Coronary Arteries. The tissue is endowed with contractile 5-HT<sub>2A</sub> receptors (Cushing and Cohen, 1993). 5-HT (3 nM to 10 μM) induced a concentration-dependent contraction in coronary arterial rings (pEC<sub>50</sub> 6.77 ± 0.03; n = 14) that was surmountably blocked by the 5-HT<sub>2A</sub> receptor antagonist ketanserin (10 nM; apparent p<sub>A<sub>2</sub></p> 8.99 ± 0.02; n = 6; data not shown). 5-HT–induced contractions were antagonized by terguride and its 2-halogenated derivatives (Fig. 4A; Table 3). Terguride (1 nM) produced only a slight dextral shift of the

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**Fig. 3.** Effects of terguride (A), 2-halogenated derivatives of terguride (B–D), and aripiprazole (E) alone or versus quinpirole (1 or 5 μM) at hD2L/Gαo receptors stably expressed in HEK293 cells. [35S]GTPγS binding is expressed as a percentage relative to the effect of the full agonist quinpirole. Points are mean ± S.E.M. of six to eight individual experiments.
ep44

CRC to 5-HT (concentration ratio \( r < 2 \), \( n = 6 \)) but induced a marked depression of the maximal response from 122 ± 8 to 44 ± 5% (\( n = 6 \)). The noncompetitive antagonist parameter \( pD^*_A \) for terguride is shown in Table 3.

2-Iodo-, 2-bromo-, and 2-chloroterguride (3 nM each) induced dextral shifts of the 5-HT curve with concentration ratios \( r > 2 \) (apparent \( pA_2 \) values, see Table 3) and reduced \( E_{\text{max}} \) from 122 ± 8 to 62 ± 9, 50 ± 3, and 38 ± 5%, respectively (Fig. 4A). In contrast to the insurmountable antagonist properties of 2-chloro-, 2-bromo-, and 2-iodoterguride, aripiprazole inhibited the contractile 5-HT effect in a surmountable manner (Fig. 4B), albeit with 40- to 135-fold lower antagonist potency than the ergolines. The apparent \( pA_2 \) of 7.12 for 0.5 \( \mu \)M aripiprazole (see Table 3) was similar to the \( pK_i \) (7.46) at cloned human 5-HT2A receptors (Shapiro et al., 2003).

Effects at Smooth Muscle \( \alpha_{2C}\)-Adrenoceptors in Porcine Pulmonary Arteries. This tissue is endowed with contractile \( \alpha_{2C}\)-adrenoceptors (Jantschak and Pertz, 2012). The \( \alpha_2 \)-adrenoceptor agonist UK-14304 (0.3 nM to 3 \( \mu \)M) induced a concentration-dependent contraction in pulmonary arterial rings after moderate precontraction with (S)/(−)-Bay K 8644 (L-type Ca\(^{2+} \) channel activator). The pEC\(_{50}\) for UK-14304 was 7.70 ± 0.09 (\( n = 10 \)). Terguride and its 2-halogenated derivatives were potent antagonists of the contractile UK-14304 response (Fig. 5A; Table 3). 2-Chloroterguride, 2-bromoterguride, and 2-iodoterguride showed higher antagonist potency compared with the antagonist effect of the parent compound terguride (Table 3). The effects of 2-chloroterguride, 2-bromoterguride, and 2-iodoterguride were nearly identical. Aripiprazole (0.5 \( \mu \)M) also blocked UK-14304-induced contractions, albeit with 400- to 1800-fold lower affinity than terguride and its derivatives (Fig. 5B, Table 3). The apparent \( pA_2 \) of 7.20 for aripiprazole (see Table 3) was in line with the \( pK_i \) (7.42) at cloned human \( \alpha_{2C}\)-adrenoceptors (Shapiro et al., 2003).

Effects at Smooth Muscle Histamine \( H_1 \) Receptors in Porcine Pulmonary Veins. Histamine (10 nM to 30 \( \mu \)M) induced a concentration-dependent contractile response in pulmonary venous rings. The pEC\(_{50}\) was 6.58 ± 0.05 (\( n = 8 \)). The effects of histamine were surmountably blocked by mepyramine (H\(_1 \) receptor antagonist; Fig. 6D). The apparent \( pA_2 \) of 8.70 ± 0.08 (\( n = 5 \)) for mepyramine argues for an involvement of \( H_1 \) receptors in the contractile response to histamine in this tissue. Terguride (1 \( \mu \)M) behaved as a low-efficacy partial agonist; the slight contractile response to terguride (5 ± 1%) was abolished by mepyramine (1 \( \mu \)M). As expected for a partial agonist, terguride (1 \( \mu \)M) inhibited the contractile histamine response (Fig. 6A). The halogenated derivatives of terguride were devoid of partial agonist effects and antagonized the histamine-induced contraction (Fig. 6B; Table 3). Aripiprazole also inhibited the histamine response but with an affinity that was 20- to 80-fold higher than that of terguride and its derivatives (Fig. 6C; Table 3). The apparent \( pA_2 \) of 7.82 for aripiprazole (see Table 3) was in line with the \( pK_i \) (7.60) at cloned human \( \alpha_{2C}\)-adrenoceptors (Shapiro et al., 2003).

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT(_{3A})</th>
<th>( \alpha_{2C} )</th>
<th>( H_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terguride</td>
<td>9.25 ± 0.09 (6)(^b)</td>
<td>9.77 ± 0.05 (6)</td>
<td>6.55 ± 0.04 (5/4)</td>
</tr>
<tr>
<td>2-Chloroterguride</td>
<td>8.76 ± 0.04 (6)</td>
<td>10.33 ± 0.08 (6)</td>
<td>6.25 ± 0.05 (4)</td>
</tr>
<tr>
<td>2-Bromoterguride</td>
<td>8.78 ± 0.05 (6)</td>
<td>10.45 ± 0.08 (6)</td>
<td>5.96 ± 0.04 (4)</td>
</tr>
<tr>
<td>2-Iodoterguride</td>
<td>8.73 ± 0.05 (6)</td>
<td>10.15 ± 0.04 (6)</td>
<td>5.91 ± 0.06 (4)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>7.12 ± 0.19 (4)</td>
<td>7.20 ± 0.10 (4)</td>
<td>7.82 ± 0.09 (4)</td>
</tr>
</tbody>
</table>

\(^a\)Apparent \( pA_2 \).

\(^b\)\( pD^*_A \), noncompetitive antagonist parameter (van Rossum, 1963).

\(^c\)\( pK_i \), partial agonist affinity, calculated from the antagonism of the histamine response by the partial agonist (Marano and Raumann, 1976).
treated rats showed hyperlocomotion compared with the vehicle group. 2-Bromoterguride (0.1 and 0.3 mg/kg) and aripiprazole inhibited amphetamine-induced locomotion. A statistically significant main effect of treatment ($F_{4,4473} = 16.0; P < 0.001$), time ($F_{1,4473} = 24.9; P < 0.001$), and interaction of the factors ($F_{44,4473} = 4.3; P < 0.001$) was found (Fig. 9).

**Discussion**

A primary goal of our study was to examine the pharmacologic properties of 2-halogenated derivatives of terguride at dopaminergic (D2), serotonergic (5-HT2A), adrenergic (a2C), and histaminergic (H1) receptors because these receptors play a role in the pathophysiology and treatment of schizophrenia (Roth et al., 2004). The effects of terguride, the parent drug of these compounds, at D2, 5-HT2A, a2C, and H1 receptors were fully in agreement with those reported by others (Newman-Tancredi et al., 2002a,b; Pertz et al., 2006; Kekewska et al., 2012).

Striatal dopaminergic hyperfunction plays a causative role in schizophrenia (Howes and Kapur, 2009; Simpson et al., 2010). At therapeutic doses, antipsychotic drugs block striatal D2 receptors. There are two isoforms of the D2 receptor: the short one (D2S) has the function of a somatodendritic autoreceptor of central dopaminergic neurons, and the long one (D2L) plays the role of a postsynaptic receptor in vivo (Khan et al., 1998). D2 receptor partial agonists such as aripiprazole, preclamol, 7-[3-(4-[2,3-dimethylphenyl]piperazinyl)propoxy]-2(1H)-quinoline (OPC-4392), and terguride can show large variations in intrinsic activity and potency at both isoforms. Depending on the assay used (e.g., adenylyl cyclase or GTP-S binding), the differential activation of signaling pathways (a phenomenon termed functional selectivity), the level of receptor expression, the host cell type, and the drug concentration, these compounds demonstrated partial agonist or antagonist properties at D2L and D2S receptors (Lawler et al., 1999; Burris et al., 2002; Newman-Tancredi et al., 2002a; Shapiro et al., 2003; Tadori et al., 2005).

In our studies, aripiprazole was devoid of agonist activity and behaved as an antagonist at hD2S receptors in the GTP-S binding assay (Fig. 1B). However, aripiprazole was a partial agonist of moderate intrinsic activity at hD2L receptors in the adenylyl cyclase assay (i.e. 0.29; see Fig. 2; Table 3). It should be noted that the measurement of cAMP levels is focused on a strongly amplified event downstream of the G protein–coupled receptor, whereas the measurement of GTP-S reflects a comparably lower amplification event close to the receptor (Strange, 2010); as a consequence, a partial agonist in the adenylyl cyclase assay may behave as an antagonist in the GTP-S binding assay. Our findings that aripiprazole caused no agonist effect at hD2L receptors expressed in CHO cells in the GTP-S binding assay is totally in line with the data reported by Shapiro et al. (2003).

The observation in our study that terguride behaved as a silent hD2L receptor antagonist and a hD2S receptor partial agonist is consistent with the pharmacologic profile of this drug in GTP-S binding studies of Newman-Tancredi et al. (2002a). It has been hypothesized that the inefficacy for the treatment of schizophrenia of D2 receptor partial agonists such as terguride, preclamol, or OPC-4392 (Benkert et al., 1995) may be based upon their appreciably high intrinsic activity at D2S receptors (Natesan et al., 2011). In our GTP-S binding studies, we could show that halogenation of terguride in the 2-position reduced the intrinsic activity by 46–68%.

**Catalepsy.** 2-Bromoterguride did not induce catalepsy in the block or grid test. All estimated mean catalepsy scores of 2-bromoterguride were <2, the level at which catalepsy is considered to begin (Wadenberg et al., 2001) (Fig. 7). However, haloperidol (0.5 mg/kg) induced cataleptic behavior both in block and grid test and prolonged descent latency compared with vehicle and/or 2-bromoterguride. The results were: block test: haloperidol versus 0.1 mg/kg 2-bromoterguride at 60 minutes ($H = 9.8, P = 0.02, df = 3$); haloperidol versus vehicle and 0.1 mg/kg 2-bromoterguride at 90 minutes ($H = 23.3, P < 0.001, df = 3$), 120 minutes ($H = 20.1, P < 0.001, df = 3$), and 150 minutes ($H = 15.6, P < 0.001, df = 3$); grid test: haloperidol versus vehicle, 0.1 and 0.3 mg/kg 2-bromoterguride at 30 minutes ($H = 16.6, P < 0.001, df = 3$); haloperidol versus vehicle and 0.1 mg/kg 2-bromoterguride at 60 minutes ($H = 24.3, P < 0.001, df = 3$), 90 minutes ($H = 25.8, P < 0.001, df = 3$), 120 minutes ($H = 23.0, P < 0.001, df = 3$), and 150 minutes ($H = 19.9, P < 0.001, df = 3$) (Fig. 7).

**Effects of 2-Bromoterguride on Spontaneous Behavior in the Open Field.** Locomotor activity was affected by 2-bromoterguride treatment. Both 2-bromoterguride doses, 0.1 and 0.3 mg/kg, decreased spontaneous locomotion ($F_{2,27} = 25.2; P < 0.001$; Fig. 8) and rearings ($F_{2,27} = 19.0; P < 0.001$; vehicle: $131 \pm 24$; 0.1 mg/kg 2-bromoterguride: $86 \pm 13$; 0.3 mg/kg 2-bromoterguride: $46 \pm 14$).

**Effects of 2-Bromoterguride on AIL.** Amphetamine-treated rats showed hyperlocomotion compared with the

![Graph A](image1)

![Graph B](image2)

**Fig. 5.** Contractile responses to UK-14304 (a2-adrenoceptor agonist) in porcine pulmonary arteries moderately precontracted with (S)-(–)-Bay K 8644 (L-type Ca2+ channel activator) in the absence or presence of terguride and its 2-halogenated derivatives (A) and aripiprazole (B). Points are mean values ± S.E.M. of four to six animals.
compared with that of the parent drug terguride (Fig. 1A; Table 2). Moreover, 2-bromoterguride showed an intrinsic activity that was half as high as that of terguride at hD2S receptors in the adenylyl cyclase assay. Most interestingly, 2-bromoterguride mimicked aripiprazole in this assay; intrinsic activity was the same for both drugs (Fig. 2; Table 2).

These data are in agreement with the hypothesis of a reciprocal relationship between intrinsic activity and antipsychotic efficacy of partial agonists in schizophrenia (Natesan et al., 2011). In addition to the effects at hD2S receptors, terguride and its derivatives behaved as potent antagonists at hD2L/Ga o receptors. The hD2L receptor preferentially couples to Ga o over each of the three other Ga i subtypes: Ga i1, Ga i2, and Ga i3 (Gazi et al., 2003). Postsynaptic D2L receptor blockade is the essential antipsychotic mechanism downstream of the primary dopaminergic abnormality (Howes and Kapur, 2009). A relatively high affinity for 5-HT2A compared with D2 receptors, the so-called 5-HT2A/D2 ratio, has been proposed as a significant but not exclusive criterion for differentiating typical and atypical antipsychotic drugs; a higher affinity for 5-HT2A over D2 receptors may improve negative symptoms and may have an impact on EPS liability (Kalkman and Loetscher, 2003; Kroeze et al., 2003).

In rodents, 5 mg/kg clozapine, a dose that showed a pronounced inhibitory effect on conditioned avoidance response, produced ~45% D2, ~65% α2A-, and ~95% α2C-adrenoceptor occupancy (Marcus et al., 2005). This is in line with observations made by Kalkman and Loetscher (2003) who argued that it is not the affinity of an antipsychotic drug for α2C-adrenoceptors per se but the relative affinity α2C/D2 that is important for efficacy in schizophrenia. According to these authors, a ratio α2C/D2 ≥ 1 suggests that α2C-adrenoceptor blockade may contribute to the therapeutic profile of an antipsychotic drug. This is the case for atypical antipsychotics such as clozapine, olanzapine, risperidone, and quetiapine (Kroeze et al., 2003).

Aripiprazole exhibited much lower α2C-adrenoceptor affinity (pA2 7.2) and therefore does not fulfill the criterion of Kalkman and Loetscher (2003).
Affinities for histamine H₁ receptors are positively correlated with weight gain among typical and atypical antipsychotic drugs (Kroeze et al., 2003). Accordingly, it has been recommended that novel antipsychotic drugs should possess a low affinity for H₁ receptors. In our studies, the affinity of aripiprazole for H₁ receptors was higher than that of terguride and its derivatives.

For our in vivo experiments, we used preclinical tests with high predictive validity, which offer valuable clues to the effect of 2-bromoterguride on central dopaminergic mechanisms. In rodents, cataleptic behavior is evident with most typical antipsychotics and is less obvious with atypical antipsychotics, and it is regarded as a predictor of EPS liability (Arnt and Skarsfeldt, 1998; Wadenberg et al., 2001; Natesan et al., 2006). Consistent with other D₂ receptor partial agonists (Semba et al., 1995; Natesan et al., 2011), 2-bromoterguride failed to produce catalepsy. Although 2-bromoterguride increased descent latency in the block and grid tests, a state of immobility was not attained. In contrast, catalepsy was induced by the typical antipsychotic drug haloperidol (positive control). In addition to the catalepsy test, we studied the effect of 2-bromoterguride on spontaneous behavior in the open field. 2-Bromoterguride decreased locomotor activity and rearings of rats. This is in agreement with other studies showing an attenuating effect of D₂ receptor partial agonists on locomotion and rearings (Svensson et al., 1991; Nordquist et al., 2008). Hence, the possibility that some effects of 2-bromoterguride observed in AIL are affected by these motor effects cannot be excluded. However, the low affinity of 2-bromoterguride for the H₁ receptor argues against an involvement of this receptor in the sedative effect.

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(Porsolt et al., 2010), a response that should readily be antagonized by typical and atypical antipsychotics by competing for postsynaptic D₂ receptors in this part of the mesolimbic DA system (Geyer and Ellenbroek, 2003). Our observation that 2-bromoterguride (0.1 and 0.3 mg/kg) inhibited AIL clearly suggests the antidopaminergic properties of this drug. Interestingly, a comparable inhibition of AIL was only achieved with a 10-fold higher dose of aripiprazole (3 mg/kg). Furthermore, inhibition of AIL confirms the in vitro characteristics of this drug. AIL clearly suggests the antidopaminergic properties of the mesolimbic DA system (Geyer and Ellenbroek, 2003). Our observation that 2-bromoterguride behaved as a potent antagonist at hD₂/L/G protein subtype. Evidence for agonist selection of G protein subtype. Eur J Pharmacol 462:33–40.

References


Fig. 9. Effect of intraperitoneal administration of 2-bromoterguride (0.1 and 0.3 mg/kg) or aripiprazole (3.0 mg/kg) on amphetamine-induced locomotion (1.5 mg/kg amphetamine at time = 0 minutes). 2-Bromoterguride and aripiprazole antagonized drug-induced hyperactivity. Data are mean ± S.E.M. of 10 (vehicle and 2-bromoterguride) or 8 animals (aripiprazole). *P < 0.05 2-bromoterguride (0.1 and 0.3 mg/kg) and aripiprazole versus amphetamine. †P < 0.05 2-bromoterguride (0.3 mg/kg) and aripiprazole versus amphetamine. 2-Br-Terg, 2-bromoterguride; Amph, amphetamine; Arip, aripiprazole; Veh, vehicle.

Wrote or contributed to the writing of the manuscript: Brosda, Hübner, Jantschak, Pertz.

Acknowledgments

The authors thank Dr. T. Paulke and M. Uwarow of the Lehr- und Versuchsanstalt für Tierzucht und Tierhaltung (Teltow-Ruhlsdorf, Germany) for providing pig hearts and lungs for the studies, and Sabine Jacobs and Aurica Kaufeld for skillful technical assistance.

Authorship Contributions

Participated in research design: Brosda, Fink, Gmeiner, Jantschak, Pertz.

Conducted experiments: Brosda, Franke, Hübner, Jantschak, Müller, Pertz.

Performed data analysis: Brosda, Pertz.

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