Synthesis and Pharmacological Evaluation of Triflate-Substituted Analogues of Clozapine: Identification of a Novel Atypical Neuroleptic

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The trifluoromethanesulfonyloxy (TfO) analogues **3** and **4** of 8-chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (clozapine, **1**) and its 2-chloro isomer (iso-clozapine, **2**), respectively, were synthesized via their OMe and OH analogues with the conventional synthetic method of the tricyclic dibenzodiazepines and evaluated pharmacologically along with their parent drugs. The binding profile of the 2-OTf analogue (**4**) is comparable to the binding profile of **1**, although the affinity for the dopamine (DA) D_2 receptors is higher (IC_{50} values are 31 nM and 330 nM for compounds **4** and **1**, respectively). Interestingly, no notable affinity for muscarinic receptors could be detected in compound **4**. On the contrary, the 8-OTf analogue **3** only displayed affinity for muscarinic M_1 receptors (IC_{50} value 35 nM) and no affinity (IC_{50} value > 500 nM) for the other receptors tested. The 10 μmol/kg sc dose, but not the 10 μmol/kg po dose, of compound **4** stimulated the output of DA. Increases of 80% and 35% in DOPAC output from the dorsal striatum were seen after sc and po administrations of 10 μmol/kg of compound **4**, respectively. Doses up to 100 μmol/kg of compound **3** had no effect on either parameter. Doses up to 100 μmol/kg of compound **4** were not cataleptogenic, but significantly decreased apomorphine-induced locomotor activity. In conclusion, compound **4** (GMC1-169) is a new clozapine-like neuroleptic candidate, which is lacking anticholinergic properties and displays a higher potency, as compared to clozapine (**1**) itself.

Introduction

It is generally believed that antipsychotic agents that are used to treat schizophrenia reduce the dopaminergic activity in the central nervous system (CNS) by blocking dopamine (DA) receptors, in particular DA D_2 receptors.[1−4] In addition to their antipsychotic efficacy, the so-called typical neuroleptics such as chlorpromazine[5,6] and haloperidol,[7] that have a high affinity for DA D_2 receptors, cause acute and chronic motor disturbances (extrapyramidal side-effects, EPS). Furthermore, a large treatment-resistant segment of the schizophrenic population receives little, if any, benefit from the use of typical neuroleptics. The effective antipsychotic clozapine (**1**, Figure 1) couples a low incidence of EPS with a high efficacy in treatment-resistant schizophrenia[8−11] and is therefore referred as an atypical neuroleptic. Still, the use of clozapine (**1**) is limited due to its propensity to induce agranulocytosis[12−14] and seizures.[15−18] In addition, the fact that clozapine (**1**) displays affinity for a vast variety of central nervous system (CNS) receptors and receptor subtypes causes a multitude of acute central and peripheral side-effects such as lack of concentration, weight gain, hypersalivation, orthostatic hypotension etc.[9,15−17,19]

Hypotheses have been proposed by several researchers attempting to account for the atypical nature of clozapine (**1**). For example, it has been suggested that (i) a favorable 5-HT_{2A}/D_2 affinity (pK_i) ratio,[10] (ii) a high affinity for DA D_4 receptors,[20,21] or (iii) a partial agonism, with a low intrinsic efficacy, at DA D_2 receptors[22] would account for the atypical nature of clozapine. Yet, it remains possible that these hypotheses each only account for a part of the beneficial clinical profile of clozapine. For example, risperidone, a 5-HT_{2A}/D_2 antagonist that is currently used in the clinic, only has a lesser propensity for side-effects in a very narrow dose-range.[16,19]

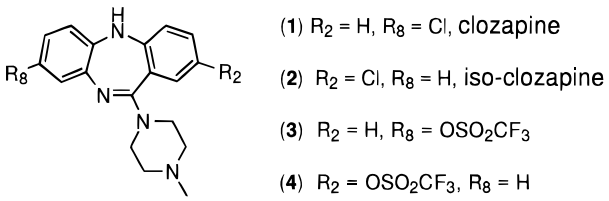

Figure 1. Structures discussed in the text.

An alternative approach to develop potential atypical neuroleptics is to slightly modify the basic 5*H*-dibenzo[*b*,*e*][1,4]diazepine skeleton of clozapine (**1**), to yield new chemical entities (NCEs), hopefully keeping the beneficial properties of clozapine (**1**) and losing some of the side-effects.[23−25] Among the newer antipsychotics that have emerged from this approach are olanzapine[26,27] and seroquel.[28] Interestingly, the binding profiles of these two compounds resemble that of clozapine (**1**) with some subtle differences. On the other hand, iso-clozapine (**2**) is known to be a potent typical neuroleptic, which has been reported to induce catalepsy in the rat at 1.8 mg/kg sc.[29] Our approach has thus been to stay even closer to the basic structure of clozapine and to investigate the effect of substitution of the halogens on the 5*H*-dibenzo[*b*,*e*][1,4]diazepine skeleton of compounds **1** and **2** by the strongly electron-withdrawing triflate group. We have compared the pharmacological effects of these new clozapine analogues **3** and **4** with the profiles of the parent drugs in the same pharmacological models.

In contrast to the reactive character of aliphatic triflates, aromatic triflate groups are known to be both chemically[30] and biologically stable.[31,32] Due to their

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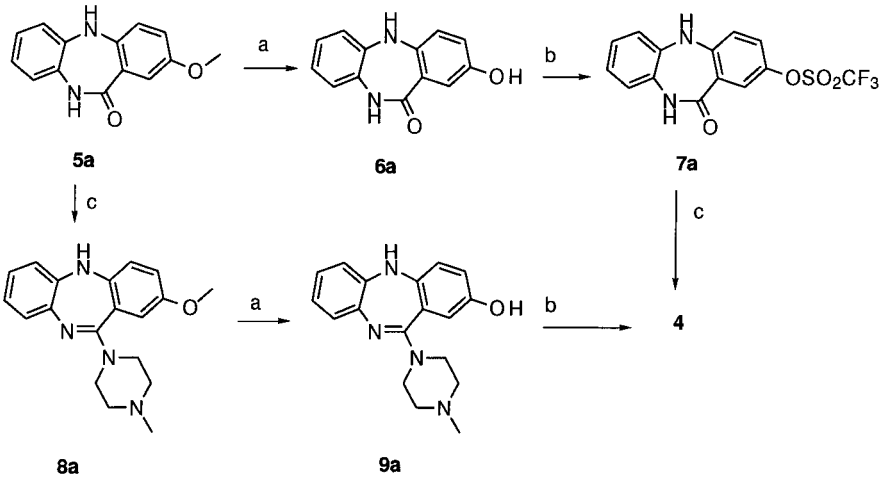
Scheme 1



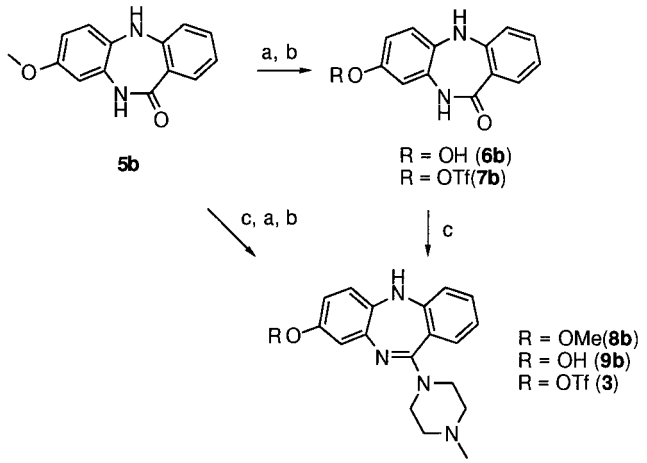
Reagents and conditions: a) $AlCl_3$, EtSH, rt, 4 h; b) $PhN(SO_2CF_3)_2$, Et_3N, CH_2Cl_2, rt, overnight; or Tf_2O, Et_3N, -78 °C; c) i, $POCl_3$, toluene, N,N-di-Me-aniline, reflux, 3 h; ii, N-Me-piperazine, toluene, reflux, 3 h.

electron withdrawing properties, they also prevent oxidation of the aromatic ring via the cytochrome P450 iso-enzyme systems. A triflate functionality on an aromatic ring system should thus be beneficial in slowing down the metabolism (e.g. hydroxylation and consequent conjugation) that otherwise would have been directed toward an aromatic ring carrying an electron-donating group like a hydroxy or methoxy group. This modification can thus be used to diminish the metabolism of a pharmacologically active compound possessing a hydroxy- or methoxy-substituted aromatic ring, provided that the triflated molecule still has an interesting pharmacological profile. The validity of this concept has recently been demonstrated by a number of centrally active triflated compounds with an improved oral bioavailability.[31−36] In this study, we wish to report a successful application of the triflate modification in the search of a new clozapine-like neuroleptic.

Chemistry

The synthesis of 2-trifluoromethanesulfonyloxy-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4] diazepine (**4**) is shown in Scheme 1 and is representative for the synthesis of both analogues. Intermediate **5a**, 2-methoxy-5,10-dihydro-11-oxo-dibenzo[*b*,*e*][1,4]diazepine, was prepared as described before.[37,38] Several trials of demethylation of **5a** by BBr_3, refluxing 48% HBr, or TMSI under a variety of conditions did not succeed. However, **5a** was successfully demethylated by aluminum chloride in ethylmercaptan at room temperature to yield **6a**, which was sulfonated by *N*-phenyltrifluoromethanesulfonimide or triflic anhydride. Intermediate **7a** was treated with phosphorus oxychloride under reflux in toluene to form the imino chloride intermediate, which was further converted to the desired compound **4** in high yield by treatment of *N*-methylpiperazine (method A). Alternatively, compound **8a**[37] could be prepared from **5a** and then demethylated by aluminum chloride in ethylmercaptan at room temperature to afford 2-hydroxy-11-(4-methly-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**9a**), which was sulfonated to give the target compound **4** (method B). It should be noted here that compound **9a** has appeared once in the literature without proper chemical documentation.[39] Among the two synthetic methods mentioned above, the first one is more efficient, giving a higher total yield.

Scheme 2



Reagents and conditions correspond to those of Scheme 1.

8-(Trifluoromethanesulfonyloxy)-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**3**), was synthesized from the appropriate starting material **5b** in a similar manner (Scheme 2). Compound **9b** was previously only identified by GC/MS as one of the metabolites of clozapine (**1**) in the urine of schizophrenic patients.[40]

Pharmacology

1. *In Vitro* Receptor Binding Study. Dopamine D_1 Receptors. Inhibition by drugs of the binding of 0.20 nM [^{3}H]SCH23390 to dopamine D_1 receptors in membranes from rat corpus striatum was determined as described by Hyttel et al.[41,42]

Dopamine D_2 Receptors. Inhibition by drugs of the binding of 0.50 nM [^{3}H]-spiperone to dopamine D_2 receptors in membranes from rat corpus striatum was described by Hyttel.[43,44]

Serotonin 5-HT_{2A} Receptors. Inhibition by drugs of the binding of 0.50 nM [^{3}H]ketanserin to 5-HT_{2A}

Table 1.[a] A Comparison of the IC_{50} Values (nM) of Haloperidol,[b] Clozapine (1),[b] Iso-clozapine (2), Compounds 3, 4, 8a, 8b, 9a, and 9b to D_1 and D_2, 5-HT_{2A}, 5-HT_{2C}, M_1, H_1 and α_1 Receptors[c]

compound	D_1	D_2	5-HT_{2A}	5-HT_{2C}	H_1	α_1	M_1
haloperidol	36	7.5	55	>1000	>1000	18	5500
1	130	330	7.8	11	23	9.2	9.4
2	11	13	12	2.9	NT[d]	64	6.0
3	930	8100	550	620	720	>1000	35
4	64	31	8	34	47	12	1200
8a	550	68	12	21	NT[d]	120	39
8b	7400	4300	160	86	NT[d]	240	19
9a	550	1300	35	81	NT[d]	260	37
9b	9800	6400	310	440	NT[d]	1200	27

[a] Results are expressed as IC_{50}-values in nM (logarithmic means). Two full concentration−response curves were measured using five concentrations of test drug in triplicate (covering 3 decades). [b] See also ref 52 (Sánchez paper). [c] IC_{50} > 860 nM for the binding of compounds **1**−**4** and haloperidol to 5-HT_{1A} receptors; not tested for the binding of compounds **8a**, **8b**, **9a**, and **9b** to 5-HT_{1A} receptors. [d] Not tested.

receptors in membranes from rat brain was determined as described by Hyttel.[44]

Serotonin 5-HT_{2C} Receptors. Inhibition by drugs of the binding of 0.50 nM [^{3}H]mesulergine to cloned rat 5-HT_{2C} receptors expressed in membranes from 3T3 cells was determined as described by Bøgesø et al.[45]

α_1-Adrenoceptors. Inhibition by drugs of the binding of 0.25 nM [^{3}H]prazosin to α_1-adrenoceptors in rat brain membranes was determined as described by Arnt et al.[46] as modified from Hyttel and Larsen.[47]

Muscarinic Cholinergic M_1 Receptors. Inhibition by drugs of the binding of 1.0 nM [^{3}H]pirenzepine (PZ) to cloned human m1 receptors expressed in membranes from CHO-K1 cells was estimated as described by Meier et al.[48]

Histamine H_1 Receptors. Inhibition by drugs of the binding of 2.0 nM [^{3}H]mepyramine to histamine H_1 receptors in rat brain membranes was determined as described by Hall et al.[49]

Serotonin 5-HT_{1A} Receptors. Inhibition by drugs of the binding of 1.0 nM [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide) to 5-HT_{1A} receptors in rat brain membranes was determined as described by Hyttel et al.[50]

Results on receptor binding study were given as IC_{50} values (nM) (Table 1). Two complete concentration−response curves were determined by using five concentrations of the test drugs in triplicate (covering 3 decades). IC_{50} values were estimated from hand-drawn log concentration−response curves or computer-assisted log−logit analysis. In a series of *n* determinations the variance of the log ratio (VAR_R) between the double determinations is determined according to the formula: $VAR_R = \Sigma (\log R_1)^2/2n$; where R_1 is the ratio and n is the number of observations. The VAR_R is equivalent to the square of the standard deviation of the log ration (SD_R^2). In case the ratio is greater than corresponding to 2 × SD_R (95% confidence interval), further determinations were performed and outliers discarded. For the binding analysis, the following SD_R's were obtained: D_1, 1.5 (n = 100); D_2, 1.5 (n = 100); 5-HT_{1A}, 1.1 (n = 100); 5-HT_{2A}, 1.4 (n = 30); 5-HT_{2C}, 1.3 (n = 100); H_1, 1.5 (n = 100); α_1, 2.0 (n = 76); M_1, 1.4 (n = 91).[48]

2. *In Vivo* Microdialysis. Male rats of a Wistar-derived strain (Harlan, Zeist, The Netherlands) weighing 275−350 g were implanted with microdialysis probes in striatum according to previously described methods.[51] Dialysis experiments were started 24−48 h after probe implantation. Probes were perfused with artificial cerebrospinbal fluid (aCSF, in mM: NaCl: 147, KCl: 3, $CaCl_2$: 1.2, and $MgCl_2$: 1.0) and extracellular levels of DA and DOPAC were quantified by HPLC with electrochemical detection as previously described.[51] After stabilization of the output values (<20% variation between samples), drugs were administered either sc (1 mL/kg) or po (2 mL/kg). Drug-effects were followed for at least 2.5 h. Data are expressed as percentages of control values, i.e., the average output of the four samples immediately preceding drug-administration is set at 100%. Drug-effects were analyzed using two-way ANOVA with repeated measures (p <0.05) followed by Dunnett's multiple comparison test (p <0.05).

3. Behavioral Pharmacology.[52] Catalepsy. Male rats of a Wistar-derived strain (Harlan, Zeist, The Netherlands) weighing 175−225 g were used. Drugs were administered sc 30 min before testing. Rats were then placed on a vertically placed grid (mesh-width 2.5 × 2.5 cm) 10 cm above the floor. The time needed to leave the grid was measured in three consecutive sessions and averaged for further analysis. Rats that needed >30 s to leave the grid were considered cataleptic.

Inhibition of Apomorphine-Induced Locomotion. Male rats of a Wistar-derived strain (Harlan, Zeist, The Netherlands) weighing 175−225 g were used. Drugs were administered 30 min before the administration of 1 mg/kg apomorphine. After administration of apomorphine, rats were transferred to an automated activity monitoring system (Automex II, Columbus, OH) and activity was measured for 30 min. Experiments were done in parallel on four different animals and per session three experimental groups and one saline-treated group were studied. Activity in the experimental groups is expressed as percentage of the locomotor activity observed in the control groups. Data were analyzed with a nonparametric Kruskal-Wallis ANOVA (p <0.05) followed by Dunnett's multiple comparison test (p <0.05).

Results and Discussion

The compounds were tested in binding assays *in vitro* for their affinities to DA D_1 and D_2, serotonin 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C}, H_1, α_1, and M_1 receptors. The receptor-binding profiles (Table 1) of compounds **3** and **4** were strikingly different. Compound **3** was found to display binding only to muscarinic M_1 receptors (IC_{50} = 35 nM) and no binding (IC_{50} >550 nM) to the other receptors tested. The binding profile of its isomer, the 2-OTf analogue **4**, is comparable to the binding profile of clozapine (**1**), although the affinity for D_2 receptors is higher (IC_{50} = 31 nM and 330 nM for compounds **4** and **1**, respectively). Interestingly, no notable affinity for muscarinic receptors could be detected in compound **4** (IC_{50} = 1200 nM), which may be of significant clinical importance for avoiding GI symptoms and cognitive disturbances. In comparison with the typical neuroleptic haloperidol, compound **4** has a lower affinity for DA D_2 receptors (IC_{50} = 7.5 nM and 31 nM, respectively) and a slightly higher affinity for 5-HT_{2A} receptors (IC_{50} = 55 nM and 8 nM, respectively). The typical neuro-

leptic iso-clozapine (**2**), on the other hand, displayed higher affinities than compound **4** for DA receptors (IC_{50} = 11 and 13 nM for D_1 and D_2 receptors, respectively), serotonin-2 receptors (IC_{50} = 12 nM and 2.9 nM for 5-HT_{2A} and 5-HT_{2C} receptors, respectively), and M_1 receptors (IC_{50} = 6.0 nM). None of the compounds tested showed any 5-HT_{1A} affinity (IC_{50} > 860 nM). Compound **4** displayed similar affinity as clozapine (**1**) for H_1 (IC_{50} = 47 nM and 23 nM, respectively) and α_1 receptors (IC_{50} = 12 nM and 9.2 nM, respectively) while typical neuroleptic haloperidol displayed high affinity for α_1 (IC_{50} = 18 nM) but not for H_1 (IC_{50} > 1000 nM). A moderate affinity of clozapine for the histamine H_3 receptor in rat cerebral cortex was recently reported.[53] Our compounds **3** and **4** were found to be inactive for H_3 receptors (K_i > 20 μM, versus K_i = 0.2 μM for clozapine) in a previous report.[54]

For a better judgment of the effect of a triflate substituent in position 2 or 8 of a neuroleptic piperazinyldibenzodiazepine, the parallel testings *in vitro* of compounds **8a**, **8b**, **9a**, and **9b** were carried out (Table 1). 2-OMe analogue **8a** had lower affinities for DA D_1 (IC_{50} = 550 nM) and D_2 receptors (IC_{50} = 68 nM), and α_1 receptors (IC_{50} = 120 nM), than compound **4**. Like compound **4** and clozapine, compound **8a** had also high affinities for the serotonin 5-HT_{2A} (IC_{50} = 12 nM) and 5-HT_{2C} receptors (IC_{50} = 21 nM). The O-demethylated analogue **9a** of **8a**, was shown losing affinity for D_2 receptors (IC_{50} = 1300 nM) dramatically. On the other hand, 8-OMe (**8b**) and 8-OH (**9b**) analogues of clozapine were inactive at DA D_1 and D_2 receptors (IC_{50} > 4300 nM). Compound **8b** displayed moderate affinities for 5-HT_{2A} and 5-HT_{2C} receptors (IC_{50} = 160 nM and 86 nM, respectively) while **9b** displayed even lower affinities (IC_{50} > 310 nM). Interestingly, all four compounds (**8a**, **8b**, **9a**, and **9b**) with an electron-donating substituent on 2- or 8-positions displayed good affinity for M_1 receptors (IC_{50} values in a range of 19−39 nM) like compound **3** bearing an electron-withdrawing triflate group on 8-position but not like their 2-OTf analogue **4**.

The compounds were further tested using *in vivo* microdialysis (Figures2 and 3). Doses up to 100 μmol/kg sc of compound **3** had no effect on either DA or DOPAC output levels. Given its low *in vitro* affinities, compound **3** was not further evaluated. In contrast, the output of DA from striatum was significantly stimulated to 220% of control values for >2.5 h after sc administration of 10 μmol/kg of compound **4** [$F(10,65) = 32.537$, $p < 0.001$; $n = 6$], but not after po administration [$F(10,32) = 2.030$, $p = 0.085$; $n = 3$]. Both sc and po administration of 10 μmol/kg of compound **4** increased the extracellular levels of DOPAC in striatum [$F(10,65) = 16.872$, $p < 0.001$; $n = 6$ and $F(10,32) = 4.181$, $p = 0.003$; $n = 3$, respectively]. The maximal increase in DOPAC after sc administration of 10 μmol/kg of compound **4** was to 180% of control values and lasted for >2.5 h, whereas the maximal increase after po administration of compound **4** was to 135% of control values and lasted for 2 h.

For comparison, clozapine (**1**), iso-clozapine (**2**), and haloperidol were also studied with *in vivo* microdialysis. Clozapine (**1**; 30 μmol/kg sc) produced a small but transient increase in the output of DA to 135% of control values, 45 min after administration [$F(10,43) = 2.743$, $p = 0.016$; $n = 4$]. A more pronounced increase to 160% of control values of striatal DOPAC levels, lasting for 1 h 45 min was observed after the sc administration of 30 μmol/kg of clozapine (**1**) [$F(10,43) = 4.592$, $p < 0.001$; $n = 4$]. Administration of 1.0 μmol/kg sc of iso-clozapine (**2**) significantly elevated the maximal DA output in striatum to 161% of control values [$F(10,65) = 6.946$, $p < 0.001$; $n = 4$] as well as the maximal output of DOPAC from striatum to 178% of control values [$F(10,65) = 17.098$, $p < 0.001$; $n = 6$]. Administration of 0.3 μmol/kg sc of haloperidol induced a significant increase to 240% of control values in extracellular levels of DA, lasting for >2.5 h [$F(10,43) = 10.280$, $p < 0.001$; $n = 4$]. Likewise, the maximal output of DOPAC from striatum was stimulated for >2.5 h to 310% of control values [$F(10,43) = 16.676$, $p < 0.001$; $n = 4$]. Thus, in comparison with clozapine (**1**) and compound **4**, both iso-clozapine (**2**) and haloperidol were biochemically much

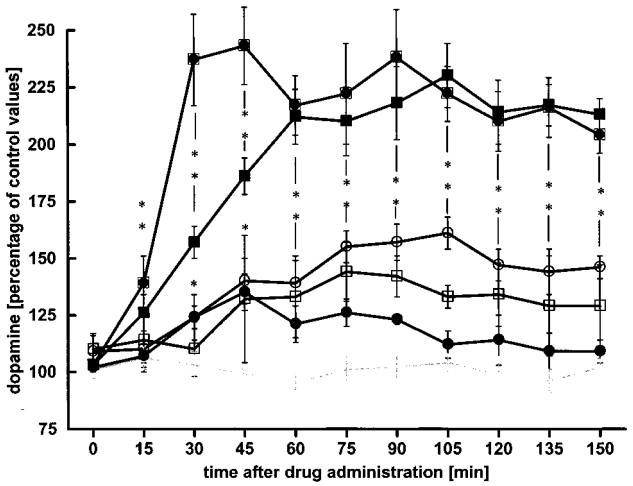


Figure 2. The effect of sc administration of saline [shaded line], 0.3 μmol/kg haloperidol [large solid circle in box], 30 μmol/kg clozapine (**1**) [●], 1.0 μmol/kg isoclozapine (**2**) [○], 10 μmol/kg compound **4** (GMC1−169) [■], and po administration of 10 μmol/kg compound **4** [□] on extracellular levels of dopamine in rat striatum. $^{**}p \leq 0.01$ vs control values (Dunnett's multiple comparison test).

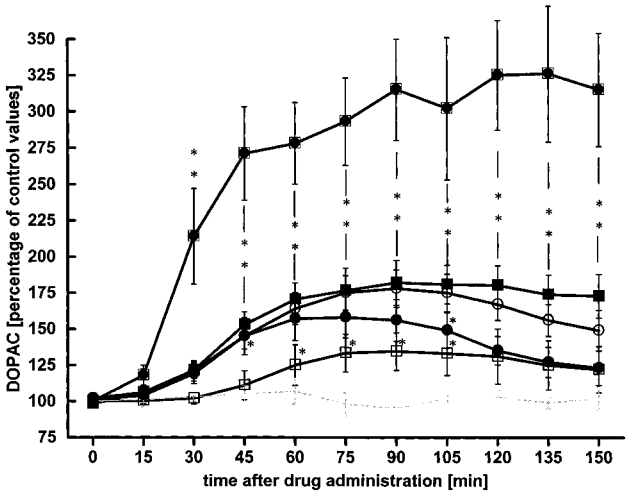


Figure 3. The effect of sc administration of saline [shaded line], 0.3 μmol/kg haloperidol [large solid circle in box], 30 μmol/kg clozapine [●], 1.0 μmol/kg isoclozapine [○], 10 μmol/kg GMC1-169 [■], and po administration of 10 μmol/kg GMC1-169 [□] on extracellular levels of DOPAC in rat striatum. $^{*}p \leq 0.05$ vs control values and $^{**}p \leq 0.01$ vs control values (Dunnett's multiple comparison test).

Table 2. The Effects of Haloperidol, Clozapine (**1**), Iso-clozapine (**2**), and Compound **4** on Apomorphine-Induced Locomotor Activity[a] and Catalepsy[b]

compound	dose (μmol/kg)	locomotor activity [% of control values ± SEM]	catalepsy [% of animals]
haloperidol	0.003	88 ± 12	0
	0.03	9 ± 3[d]	75
	0.3	1 ± 0[d]	100
1	10	98 ± 7	0
	100	7 ± 1[d]	0
2	0.3	114 ± 15	0
	1.0	82 ± 4	0
	3.0	65 ± 6	0
	10	3 ± 1[d]	25
	30	3 ± 1[d]	75
4	3	87 ± 7	NT[c]
	10	102 ± 7	0
	30	22 ± 8[d]	NT
	100	8 ± 2[d]	0

[a] Drugs were administered 30 min before 1 mg/kg apormorpine, and locomotor activity was recorded for 30 min. [b] Animals that remained on a vertical grid for >30 s were judged cataleptic. [c] Not tested. [d] Significantly different from control ($p < 0.05$).

more potent and efficient and also had a quicker onset of action as dopamine antagonists.

In contrast to haloperidol and iso-clozapine (**2**), which induced catalepsy in 75% of the rats tested at the sc doses of 0.03 and 30 μmol/kg, respectively, neither compound **4** nor clozapine (**1**) were cataleptogenic at doses up to 100 μmol/kg sc (Table 2). Still, a significant dose-related antagonism of apomorphine-induced locomotor activity was observed after the sc administration of both compound **4** (30 μmol/kg and 100 μmol/kg) [$H(4,n = 26) = 16.952$, $p = 0.002$] and clozapine (**1**; 100 μmol/kg) [$H(2,n = 15) = 8.324$, $p = 0.006$]. Administration of a cataleptogenic sc dose of 0.03 μmol/kg haloperidol decreased apomorphine-induced locomotor activity to the same extent as 100 μmol/kg sc of compound **4** or clozapine (**1**). The corresponding dose of iso-clozapine (**2**) is 10 μmol/kg sc, at which dose 25% of the rats displayed catalepsy according to the definition used in this study. In a previous study by Schmutz, iso-clozapine (**2**) was shown to be cataleptogenic at a dose of 1.8 mg/kg sc (5.5 μmol/kg sc).[29]

From the present pharmacological evaluations of compounds **3** and **4**, and with respect to the potential antipsychotic effects of these compounds, it is clear that relatively large electron-withdrawing groups are well tolerated at the 2 position, but not at the 8 position, of the 5*H*-dibenzo[*b*,*e*][1,4]diazepine skeleton. Thus, the 8-TfO clozapine analogue **3** only displayed muscarinic M_1 binding affinity, whereas the 2-TfO iso-clozapine analogue **4** resembled the atypical neuroleptic clozapine (**1**) itself, both *in vitro* and *in vivo*. In contrast to haloperidol and iso-clozapine (**2**), but like clozapine (**1**), compound **4** did not induce catalepsy, even at doses that fully blocked apomorphine-induced locomotor activity. This promising finding may indicate that the electronic character of the aromatic ring system is influencing not only the potency but also the profile, typical or atypical, of a dibenzodiazapine compound as a potential neuroleptic. Compound **4** behaved as a DA antagonist *in vivo*, since both DA and DOPAC levels in striatum increased upon its administration and the apomorphine-induced locomotor activity was antagonized in a dose-related fashion. Potentially more important, however, may be the fact that compound **4** is at least three times more potent than clozapine (**1**) in the models used. This may indicate that a lower dose of **4**, as compared to the relatively high doses used in clozapine (**1**) drug therapy, may be sufficient for a good clinical response. This may also imply less side-effects of compound **4**, as compared to those of clozapine (**1**) itself, in particular those emanating from the high muscarinic subtype affinities of clozapine (**1**).

In conclusion, compound **4** (GMC1-169) represents a novel analogue based upon substitution on the intact 5*H*-dibenzo[*b*,*e*][1,4]diazepine skeleton of clozapine (**1**) by a triflate group, leading to an *in vitro* and *in vivo* pharmacological profile commensurate with an atypical neuroleptic. More importantly, its lack of anticholinergic properties makes this drug an interesting addition to the family of atypical antipsychotic drugs.

Experimental Section

General. Melting points were determined on a Electrothermal digital melting point apparatus and are uncorrected. IR spectra were obtained on a ATI-Mattson spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini 200 NMR spectrometer. Chemical shifts are given in δ units (ppm) and relative to TMS or deuterated solvent. Coupling constants (J) are given in Hz. Mass spectra were obtained on a Unicam 610/Automass 150 GC-MS system or on a Finnegan 3300 system. Elemental analyses were performed in the Microanalytic laboratory, University of Groningen, and were within 0.4% of theoretical values. Merck silica gel (Kieselgel 60, 70−230 mesh) was used for flash chromatography. Chemicals used were either commercially available (Aldrich) and used without further purification, or prepared according to the references indicated.

2-Hydroxy-5,10-dihydro-11-oxo-dibenzo[*b*,*e*][1,4]-diazepine (6a). 2-Methoxy-5,10-dihydro-11-oxo-dibenzo[*b*,*e*]-[1,4]diazepine (**5a**)[37,38] (600 mg, 2.5 mmol) in EtSH (4 mL) was treated with $AlCl_3$ (1.6 g) with stirring at room temperature for 4 h. The mixture was quenched with ice−water (20 mL) and then 4 N aq HCl (10 mL). The solution was extracted with chloroform (2 × 20 mL) and ethyl acetate (2 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The solid residue was recrystallized from ethyl acetate to afford 505 mg (88%) of the title compound as crystals: mp 280 °C, IR (KBr) 3363, 3231, 3172, 3040, 1636, 1494 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.79 (s, 1 H), 7.41 (s, 1 H), 7.06 (s, 1 H), 6.7−6.9 (m, 7 H) ppm; ^{13}C NMR (DMSO-d_6) δ 168.3, 151.9, 143.0, 141.9, 130.4, 124.8, 124.5, 122.8, 121.6, 121.0, 120.6, 119.8, 117.2 ppm; MS (CI, NH_3) m/z 227 (M + 1). Anal. ($C_{13}H_{10}N_2O_2$) C, H, N.

2-(Trifluoromethanesulfonyloxy)-5,10-dihydro-11-oxo-dibenzo[*b*,*e*][1,4]diazepine (7a). 2-Hydroxy-5,10-dihydro-11-oxo-dibenzo[*b*,*e*][1,4]diazepine (**6a**) (400 mg, 1.77 mmol) in dioxane (10 mL) was treated with *N*-phenyltrifluoromethanesulfonimide (800 mg) in the presence of triethylamine (2 mL) with stirring at room-temperature overnight. After evaporation, the residue was purified by flash chromatography (SiO_2, hexane:ethyl acetate, 9:1 and then 4:1, as eluents). Recrystallization from hexane:ethyl acetate (10:1) afforded 380 mg (60%) of the title compound as light yellow crystals: mp 200−201 °C; IR (KBr) 3367, 3321, 3246, 3039, 1630 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1 H), 7.85 (d, 1 H, J = 3 Hz), 7.24 (m, 1 H), 7.0−6.7 (m, 5 H), 5.6 (s, 1 H) ppm; MS (EI) m/z 308 (M^+). Anal. ($C_{14}H_9N_2O_4SF_3$) C, H, N.

2-(Trifluoromethanesulfonyloxy)-11-(4-methylpiperazino)-5*H*-dibenzo[*b*,*e*][1,4] diazepine (4). Method A. 2-(Trifluoromethanesulfonyloxy)-5,10-dihydro-11-oxo-dibenzo-[*b*,*e*][1,4]diazepine (**7a**) (260 mg, 0.72 mmol), phosphorus oxychloride (4 mL), toluene (10 mL), and *N*,*N*-dimethylaniline (0.5 mL) were combined and heated to reflux for 3 h. The mixture was evaporated under vacuum to afford the imino chloride intermediate, which was used in the next step without further purification.

The above imino chloride in toluene (10 mL) was treated with *N*-methylpiperazine (3 mL) under reflux for 3 h. After evaporation, the residue was taken up with chloroform, washed with 2 N aq NaOH, and purified by flash chromatography (SiO_2, eluting with 1:1 hexane:ethyl acetate and then pure ethyl acetate) and recrystallized from hexane:ethyl acetate (15:1) to afford 230 mg (73%) of the title compound: mp 160 °C; IR (KBr) 3338, 1612 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3−6.7 (m, 7 H), 5.05 (s, 1 H), 3.42 (m, 4 H), 2.53 (m, 4 H), 2.37 (s, 3 H) ppm; MS (EI) *m/z* 440 (M^+). Anal. ($C_{19}H_{19}N_4O_3SF_3$) C, H, N.

2-Methoxy-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (8a). 2-Methoxy-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4] diazepine (**8a**) was prepared from intermediate **5a** in a similar manner described for the synthesis of compound **4** (method A) above, yielding the product as crystals (80%): mp 75−77 °C (lit.[37] mp 78 °C); ^1H NMR ($CDCl_3$) δ 7.10−6.68 (m, 7 H), 4.78 (s, 1 H), 3.73 (s, 3 H), 3.48 (brs, 4 H), 2.52 (brs, 4 H), 2.35 (s, 3 H) ppm; ^{13}C NMR ($CDCl_3$) δ 162.6, 156.1, 146.8, 143.0, 141.0, 127.8, 125.1, 124.8, 124.5, 121.6, 119.8, 118.4, 115.4, 56.4, 55.8, 46.9 ppm; MS (EI) *m/z* 322 (M^+).

2-Hydroxy-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (9a). 2-Methoxy-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (**8a**) (200 mg, 0.62 mmol) in EtSH (3 mL) was treated with $AlCl_3$ (1.5 g) with stirring at room temperature for 4 h. The mixture was quenched with ice−water (30 mL). The p*H* value of the solution was adjusted to 8 with 2 N aq NaOH. The solution was extracted carefully with chloroform (4 × 30 mL). The organic layers were combined, dried over $MgSO_4$, and filtered, and the solvent was evaporated. The light yellow solid residue was recrystallized from ethyl acetate to afford 140 mg (73%) of the title compound as crystals: mp 266 °C, IR (KBr) 3433, 3306, 2925, 2855, 1599, 1561, 1460 cm^{-1}; ^1H NMR (CD_3OD) δ 7.0−6.6 (m, 8 H), 3.47 (m, 4 H), 2.66 (m, 4 H), 2.41 (s, 3 H) ppm; MS (EI) *m/z* 308 (M^+). Anal. ($C_{18}H_{20}N_4O$) C, H, N.

2-(Trifluoromethanesulfonyloxy)-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (4). Method B. 2-Hydroxy-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (**9a**) (180 mg, 0.58 mmol) in CH_2Cl_2 (10 mL) was treated *N*-phenyltrifluoromethanesulfonimide (300 mg, 0.83 mmol) in the presence of triethylamine (1 mL) with stirring at room-temperature overnight. After evaporation, the residue was purified by flash chromatography (SiO_2; hexane:ethyl acetate, 1:1; and then ethyl acetate as eluents). Recrystallization afforded 200 mg (78%) of the title compound as crystals (identical with the product **4** achieved with method A above).

Preparation of compound **3** was conducted in a manner (either of methods A and B) similar to that described above for compound **4**.

8-Hydroxy-5,10-dihydro-11-oxodibenzo[*b,e*][1,4]diazepine (6b): mp 243 °C; IR (KBr) 3358, 3231, 3068, 1641 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.75 (s, 1 H), 9.10 (s, 1 H), 7.61 (d, 1 H, J = 6.6 Hz), 7.49 (s, 1 H), 7.28 (t, 1 H, J = 6.6 Hz), 6.7−6.9 (m, 3 H), 6.3−6.4 (m, 2 H) ppm; ^{13}C NMR (DMSO-d_6) δ 168.6, 153.6, 151.8, 133.3, 132.3, 132.0, 131.1, 123.1, 120.8, 120.6, 119.1, 111.4, 108.1 ppm; MS (CI, NH_3) *m/z* 227 (M + 1).

8-(Trifluoromethanesulfonyloxy)-5,10-dihydro-11-oxodibenzo[*b,e*][1,4]diazepine (7b): mp 188−190 °C; IR (KBr) 3327, 3244, 3190, 3117, 3085, 3031, 2975, 1648, 1608, 1516, 1482, 1424, 1387, 1243, 1214, 1142, 978, 898, 861, 812, 780, 769 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.83 (s, 1 H), 6.9−7.4 (m, 6 H), 7.51 (dt, 1 H, *J* = 1.6, 8.2 Hz), 8.13 (dd, 1 H, *J* = 1.6, 8.2 Hz) ppm; MS (CI, NH_3) 359 (M + 1). Anal. ($C_{14}H_9N_2O_4SF_3$) C, H, N.

Compound **9b**[39] was obtained from **8b**[37,38] in a similar manner as described for the preparation of **9a**: mp 249 °C; IR (KBr) 3420, 3306, 2925, 2855, 1599, 1561, 1460 cm^{-1}; ^1H NMR (CD_3OD) δ 7.33−7.26 (m, 2 H), 7.02−6.96 (m, 2 H), 6.67 (d, 1 H, *J* = 8.5 Hz), 6.47 (d, 1 H, *J* = 2.5 Hz), 6.35 (dd, 1 H, *J* = 8.5, 2.5 Hz), 3.41(br s, 4 H), 2.55 (br s, 4 H), 2.34 (s, 3 H) ppm; ^{13}C NMR (CD_3OD) δ 164.9, 156.3, 154.8, 142.2, 137.0, 133.1, 131.2, 124.3, 123.5, 120.9, 113.9, 111.9, 55.7, 48.2, 46.0 ppm; MS (EI) *m/z* 308 (M^+).

2-(Trifluoromethanesulfonyloxy)-11-(4-methylpiperazino)-5*H*-dibenzo[*b,e*][1,4]diazepine (3): mp 133−134 °C; IR (KBr) 3340, 1614 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.4−6.6 (m, 7 H), 5.03 (s, 1 H), 3.49 (m, 4 H), 2.53 (m, 4 H), 2.36 (s, 3 H) ppm; MS (EI) *m/z* 440 (M^+). Anal. ($C_{19}H_{19}N_4O_3SF_3$) C, H, N.

Materials and Methods for *In Vivo* Experiments. Male rates of a Wistar-derived strain (Harlan, Zeist, The Netherlands) weighing 275−350 g were used for the dialysis experiments. Until surgery for microdialysis probe implantation, the rats were housed in groups of six animals in plastic cages (70 × 50 × 20 cm) under conditions of constant temperature (20 °C) and humidity with lights on 6:30 and lights off 17:00. Food and water was available ad libitum. After surgery the rats were housed individually in Plexiglas cages (25 × 25 × 30 cm) also with free access to food and water. Animal procedures were conducted in accordance with guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals* and all protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

Drug Treatments. The following drugs were used: haloperidol (Sigma, St. Louis, MO), clozapine (RBI, Natick, MA), iso-clozapine (synthesized in our lab), compounds **3** and **4** (GMC1-169) (synthesized as described in the preceding chemistry experiments). Solutions of all drugs were freshly prepared before drug-administration: a 10 μmol/mL solution was prepared in 5% aqueous glacial acetic acid and diluted subsequently to 10 nmol/L in saline. Drugs were administered sc or po.

Surgery and Microdialysis Experiments. The microdialysis probes used in the present investigation were of a vertical, concentric design. The exposed tip of the dialysis membrane was 4 mm. The dialysis tube (i.d.: 0.22 mm; o.d.: 0.31 mm) was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). The microdialysis probes were implanted at the following coordinates: AP +0.7, ML ±3.0 relative to bregma, and V −6.0 below dura (striatum, bilateral) and AP +2.5, ML ±1.4 relative to bregma. The rats were anesthetized with chloral hydrate (400 mg/kg), and during surgery, lidocaine HCl (6% in saline, brought to p*H* = 6.0 with 1 N NaOH) was used as an adjuvant local anesthetic. Probes were secured to the skull with two set-screws and fast-curing dental cement.

Microdialysis experiments were carried out 24−48 h after implantation of the probe. Samples were collected on-line every 15 min in a 50 μL sample loop of an HPLC system. In brief, the inlet of the microdialysis probe was connected to a piece of polyethylene tubing (length, 45 cm; inner diameter, 0.28 mm) whereas the outlet of the microdialysis probe was connected to a piece of peek tubing (length, 45 cm; inner diameter, 0.12 mm). The inlet tube was connected to the perfusion pump, and the peek tube directly into the injection valve of the HPLC apparatus. The connection with the HPLC equipment introduced a lag time of about 8 min, for which the presented data are corrected. With the help of an electronic timer, the injection valve was held in the load position for 15 min, during which time the sample loop was filled with dialysate. The valve was then switched automatically to the inject position for 15 s. This procedure was repeated every 15 min, which was the time needed to record a complete chromatogram. The perfusion was carried out with an aCSF solution at a flow rate of 1.5 μL/min using a Carnegie CMA (Stockholm, Sweden) perfusion pump. The composition of the aCSF solution was (in mmol/L): NaCl, 147.0; KCl, 4.0; $CaCl_2$, 1.2; and $MgCl_2$, 1.0. Drugs were administered when baseline output of DA was stable (i.e. less than 20% variation between the samples). Rats were randomly assigned to either a saline group, or one of the drug-treatment groups. After finishing the experiment, the rat was terminated with an overdose of pentothal and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were cut, and dialysis probe placement was verified with the help of the atlas of Paxinos and Watson.

Analysis of the Dialysates. Dopamine was quantified by HPLC with electrochemical detection. A Pharmacia LKB 2150 pump was used in conjunction with an electrochemical detector (ESA, Coulochem). A Supelco LC18DB column (length 15 cm

and i.d. 4.6 mm) filled with reverse-phase C_{18} 5 μm material was used. The mobile phase consisted of a mixture of 0.1 mol/L of sodium acetate adjusted to a p*H* 4.1 with acetic acid, 1.8 mmol/L of 1-heptanesulfonic acid, 0.3 mmol/L of Na_2EDTA, and 120 mL methanol/L and was delivered at a flow rate of 0.7 mL/min.

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References

(1) Carlsson, A.; Lindqvist, M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normethanephrine in mouse brain. *Acta Pharmacol. Toxicol.* **1963**, *20*, 140−144.

(2) Creese, I.; Burt, D.; Snyder, S. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **1976**, *192*, 481−483.

(3) Seeman, P.; Lee, T.; Choa-Wong, M.; Wong, K. Antipsychotic drug dose and neuroleptic/dopamine receptors. *Nature* **1976**, *261*, 717−719.

(4) Richelson, E.; Nelson, A. Antagonism by neuroleptics of neurotransmitter receptors of normal human brain *in vitro*. *Eur. J. Pharmacol.* **1984**, *103*, 197−204.

(5) Delay, J.; Deniker, P.; Harl, J.-M. Traitment des etats d'excitation et d'agitation par une methode medicamenteuse derivée de l'hibernotherapie. *Ann. Med. Psychol.* **1952**, *110*, 267−273.

(6) Deniker, P. Discovery of the clinical use of neuroleptics. In *Discoveries in Pharmacology*, Vol 1: Psycho- and neuropharmacology; Parnam, M., Bruinvels, J., Eds.; Elservier: Amsterdam, 1983; pp 163−180.

(7) Janssen, P.; Van Bever, W. Structure−activity relationships of the butyrophenones and diphenylbutylpiperidines. In *Handbook of psychopharmacology*; Iversen, L., Iversen S., Snyder, S. H., Eds.; Plenum Press: New York, 1978; Vol. 10, pp 1−36.

(8) Kane, J.; Honigfeld, G.; Singer, J.; Meltzer, H. Clozapine for the treatment-resistant schizophrenic. *Arch. Gen. Psychiatry* **1988**, *45*, 789−796.

(9) Kane, J. Newer antipsychotic drugs. A review of their pharmacology and therapeutic potential. *Drugs* **1993**, *46*, 585−593.

(10) Meltzer, H.; Matsubara, S.; Lee, J. C. Classification of typical and antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin-2 p*K*i values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238−246.

(11) Baldessarini, R.; Frankenburg, F. Clozapine: a novel antipsychotic agent. *New Engl. J. Med.* **1991**, *324*, 746−754.

(12) Idänpäan-Heikkilä, J.; Alhava, E.; Olkinuora, M.; Palva, P. Agranulocytosis during treatment of clozapine. *Eur. J. Clin. Pharmacol.* **1977**, *11*, 193−198.

(13) Lieberman, J. A.; Johns, C. A.; Kane, J. M.; Rai, K.; Pisciotta, A. V.; Saltz, B. L.; Howard, A. Clozapine-induced agranulocytosis: non-cross-reactivity with other psychotropic drugs. *J. Clin. Psychiatry* **1988**, *49*, 271−277.

(14) Alvir, J. M. J.; Lieberman, J. A.; Safferman, A.; Schwimmer, J. L.; Schaaf, J. A. Clozapine-induced agranulocytosis: incidence and risk factors in the United States. *New Engl. J. Med.* **1993**, *329*, 162−167.

(15) Buchanan, R. W. Clozapine: efficacy and safety. *Schizophrenia Bull.* **1995**, *21*, 579−591.

(16) Casey, D. E. Side effect profiles of new antipsychotic agents. *J. Clin. Psychiatry* **1996**, *57*, 40−52.

(17) Lindström, L. H. The effect of long-term treatment with clozapine in schizophrenia: a retrospective study in 96 patients treated with clozapine for up to 13 years. *Acta Psychiatrica Scand.* **1988**, *77*, 524−529.

(18) Wilson, W. H.; Claussen, A. M. Seizures associated with clozapine treatment in a state hospital. *J. Clin. Psych.* **1994**, *55*, 184−188.

(19) Leysen, J. E.; Gommeren, W.; Eens, A.; de Chaffoy de Courcelles, D.; Stoof, J. C.; Janssen, P. A. Biochemical profile of risperidone, a new antipsychotic. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 661−670.

(20) Van Tol, H. H. M.; Bunzow, J. R.; Guan, H.-C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of a gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. *Nature* **1991**, *350*, 610.

(21) Seeman, P.; Guan, H. C.; Van Tol, H. H. M. Dopamine D_4 receptors evaluated in schizophrenia. *Nature* **1993**, *365*, 441−445.

(22) Jackson, D. M.; Mohell, N.; Bengtsson, A.; Malmberg, Å. Why does clozapine stimulate the motor activity of reserpine-pretreated rats when combined with a dopamine D_1 receptor agonist? *Eur. J. Pharmacol.* **1995**, *282*, 137−144.

(23) Liégelois, J.-F. F.; Rogister, F. A.; Bruhwyler, J.; Damas, J.; Nguyen, T. P.; Inarejos, M.-O.; Chleide, E. M. G.; Mercier, M. G. A.; Delarge, J. E. Pyridobenzoxazepine and pyridobenzothiazepine derivatives as potential central nervous system agents: Synthesis and neurochemical study. *J. Med. Chem.* **1994**, *37*, 519−525.

(24) Liégelois, J.-F. F.; Bruhwyler, J.; Rogister, F. A.; Delarge, J. E. Diarylazepine derivatives as potential atypical neuroleptic drugs: Recent Advances. *Curr. Med. Chem.* **1995**, *1*, 471−501.

(25) Phillips, S. T.; de Paulis, T.; Baron, B. M.; Siegel, B. W.; Seeman, P.; Van Tol, H. H. M.; Guan, H.-C.; Smith, H. E. Binding of 5*H*-dibenzo[b,e][1,4]diazepine and chiral 5*H*-dibenzo[a,d]cycloheptene analogues of clozapine to dopamine and serotonin receptors. *J. Med. Chem.* **1994**, *37*, 2686−2696.

(26) Fuller, R. W.; Snoddy, H. D. Neuroendocrine evidence for antagonism of serotonin and dopamine receptors by Olanzapine (LY-170, 053), an antipsychotic drug candidate. *Res. Commun. Chem. Pathol. Pharmacol.* **1992**, *77*, 87−93.

(27) Moore, N. A.; Calligaro, D. O.; Wong, D. T.; Bymaster, F.; Tye, N. C. The pharmacology of olanzapine and other new antipsychotic agents. *Curr. Opin. Invest. Drugs* **1993**, *2*, 281−293.

(28) Goldstein, J. M.; Arvanitis, L. A. ICI 204,636 (Seroquel): A dibenzothiazepine atypical antipsychotic. Review of preclinical pharmacology and highlights of Phase II clinical trials. *CNS Drug Rev.* **1995**, *1*, 50−73.

(29) Schmutz, J. Neuroleptic piperazinyl-dibenzo-azepines. Chemistry and structure−activity relationships. *Arzneim.-Forsch.* **1975**, *25*, 712−720.

(30) Streitweiser, A.; Dafforn, A. Solvolysis of aryl trifluoromethanesulfonates. *Tetrahedron Lett.* **1976**, *18*, 1435−1438.

(31) Barf, T. Ph.D. Thesis, University of Groningen, The Netherlands, *The medicinal chemistry of aryl triflates as applied to 5-HT_{1A} and 5-HT_{1D} receptor ligands*. ISBN 90 367 0681 5; NUGI 746.

(32) Barf, T.; DeBoer, P.; Wikström, H.; Peroutka, S.; Svensson, K.; Ennis, M.; Ghazal, N.; McGuire, J.; Smith, M. 5-HT_{1D} receptor agonist properties of novel 2-[5-(trifluoromethyl-sulfonyloxy)-indolyl]ethylamines and their use as synthetic intermediates. *J. Med. Chem.* **1996**, *39*, 4717−4726.

(33) Sonesson, C.; Waters, N.; Svensson, K.; Carlsson, A.; Smith, M. W.; Piercey, M. F.; Meier, E.; Wikström, H. Substituted 3-phenylpiperidines: new centrally acting dopamine autoreceptor antagonists. *J. Med. Chem.* **1993**, *36*, 3188−3196.

(34) Sonesson, C.; Boije, M.; Svensson, K.; Ekman, A.; Carlsson, A.; Romero, A. G.; Martin, I. J.; Duncan, J. N.; King, L. J.; Wikström, H. Orally active central dopamine and serotonin receptor ligands: 5-, 6-, 7-, and 8-[[(trifluoromethyl)sulfonyl]oxy]-2-(di-*n*-propylamino) tetralins and the formation of active metabolites *in vivo*. *J. Med. Chem.* **1993**, *36*, 3409−3416.

(35) Sonesson, C.; Barf, T.; Nilsson, J.; Dijkstra, D.; Carlsson, A.; Svensson, K.; Smith, M. W.; Martin, I. J.; Duncan, J. N.; King, L. J.; Wikström, H. Synthesis and evaluation of pharmacological and pharmacokinetic properties of monopropyl analogues of 5-, 7- and 8-trifluoromethylsulfonyloxy-2-aminotetralins. *J. Med. Chem.* **1995**, *38*, 8, 1319−1329.

(36) Haadsma-Svensson, S. R.; Smith, M. W.; Lin, C. H.; Duncan, J. N.; Sonesson, C.; Wikström, H.; Waters, N.; Carlsson, A.; Svensson, K. Synthesis and biological activity of C-5 modified derivatives of (+)-AJ76 and (+)-UH232: increased dopamine D3 receptor preference and improved pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 689−694.

(37) Hunziker, F.; Lauener, H.; Schmutz, J. 11-Dialkylamino-5-dibenzo[b,e][1,4] diazepines. Patent No. Fr. 1,334,944 (CI. A 61k, C 07d). Assignee: Wander, A. A.-G. **1963** (*Chem. Abstr.* **1961**, *61*, 3127d).

(38) Hunziker, F.; Lauener, H.; Schmutz, J. Zur chemie und Pharmakologie von in 10-stellung basisch substituierten 5-Dibenzo-[b,e][1,4]-diazepin-derivaten. *Arzneim.-Forsch.* **1963**, *13*, 324−328.

(39) Gerson, S. L.; Arce, C.; Meltzer, H. Y. N-Demethylclozapine: a clozapine metabolite that suppresses hemopoiesis. *Br. J. Haematol.* **1994**, *86 (3)*, 555−561.

(40) Stock, B.; Spiteller, G.; Heipertz, R. Exchange of aromatically bound halogens for hydroxy and methylthio groups in metabolizing clozapine in man. *Arzneim.-Forsch.* **1977**, *27*, 7 (5), 982−990.

(41) Hyttel, J. Preferential labeling of adenylate cyclase coupled dopamine receptors with thioxanthene neuroleptics. In *Advances in Dopamine Research: Advances in the Biosciences;* Kohsaka, M., Shohmori, T., Tsukada, Y., and Woodruff, G. N., Eds.; Pergamon Press Ltd.: Oxford, 1982; Vol 37., pp 147−152.

(42) Hyttel, J.; Arnt, J. Characterization of binding of [^{3}H]SCH 23390 to dopamine D_1 receptors. Correlation to other D_1 and D_2 measures and effect of selective lesions. *J. Neurol. Transm.* **1987**, *68*, 171−189.

(43) Hyttel, J. Effect of prolonged treatment with neuroleptics on dopamine D_1 and D_2 receptor density in corpus striatum in mice. *Acta Pharmacol. Toxicol.* **1986**, *59*, 387−391.

(44) Hyttel, J. Age related decrease in the density of dopamine D_1 and D_2 receptors in corpus striatum of rats. *Pharmacol. Toxicol.* **1987**, *61*, 126−129.

(45) Bøgesø, K. P.; Arnt, J.; Frederiksen, K.; Hansen, H. O.; Hyttel, J.; Pedersen, H. Enhanced D_1 affinity in a series of piperazine ring substituted 1-piperazino-3-arylindans with potential atypical antipsychotic activity. *J. Med. Chem.* **1995**, *38*, 4380−4392.
(46) Arnt, J.; Lembøl, H. L.; Meier, E.; Pedersen, H. Discriminative stimulus properties of the muscarinic receptor agonists Lu 26-046 and O-Me-THPO in rats. Evidence for involvements of different muscarinic receptor subtypes. *Eur. J. Pharmacol.* **1992**, *218*, 139−169.
(47) Hyttel, J.; Larsen, J. J. Neurochemical profile of Lu 19-005, a potent inhibitor of uptake of dopamine, noradrenaline and serotonin. *J. Neurochem.* **1985**, *44*, 1615−1622.
(48) Meier, E.; Frederiksen, K.; Nielsen, M.; Lembøl, H. L.; Pedersen, H.; Hyttel, J. Pharmacological *In Vitro* Characterization of the Arecoline Bioisostere, Lu25-109-T, a Muscarinic Compound with M_1-Agonistic and M_2/M_3-Antagonistic Properties. *Drug Dev. Res.* **1997**, *40*, 1−16.
(49) Hall, H.; Ögren, S.-O. Effects of antidepressant drugs on histamine H_1 receptors in the brain. *Life Sci.* **1984**, *34*, 597−605.
(50) Hyttel, J.; Bøgesø, K.; Lembøl, H. L.; Larsen, J.; Meier, E. Neurochemical profile *in vitro* of irendalone: A 5-HT_2-receptor antagonist. *Drug. Dev. Res.* **1988**, *15*, 389−404.
(51) DeBoer, P.; Enrico, P.; Wright, J.; Wise, L.; Timmerman, W.; Moor, E.; Dijkstra, D.; Wikström, H.; Westerink, B. H. C. Characterization of the effect of dopamine D_3 receptor stimulation on locomotion and striatal dopamine levels. *Brain Res.* **1997**, *758*, 83−91.
(52) Sánchez, C.; Arnt, J.; Dragsted, N.; Hyttel, J.; Lembøl, H. L.; Meier, E.; Perregaard, J.; Skarsfeldt, T. Neurochemical and *in vivo* pharmacological profile of Sertindole, a limbic-selective neuroleptic compound. *Drug Dev. Res.* **1991**, *22*, 239−250.
(53) Kathmann, M.; Schlicker, E.; Gothert, M. Intermediate affinity and potency of clozapine and low affinity of other neuroleptics and of antidepressants at H_3 receptors. *Psychopharmacology* **1994**, *116*, 464−468.
(54) Alves-Rodrigues, A.; Leurs, R.; Willems, E.; Timmerman, H. Binding of clozapine Metabolites and analogues to the histamine H_3 receptor in rat brain cortex. *Arch. Phar. Pharm. Med. Chem.* **1996**, *329*, 413−416.

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