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Synthesis and pharmacological evaluation of 11-(1,6-dimethyl-1,2,3,6-tetrahydropyridin-4-yl)-5*H*-dibenzo[b,e][1,4]diazepines with clozapine-like receptor occupancy at dopamine D_1/D_2 receptor

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ABSTRACT

Clozapine-like compound without agranulocytosis risk is need to cure the treatment resistant schizophrenia (TRS). We discovered (*S*)-**3** as Clozapine-like dopamine D_2/D_1 receptor selectivity and improved reactive metabolites formation profile by the modification of piperazine moiety in Clozapine. The optimization of (*S*)-**3** gave compound **5** to be best compound (approximately 10-fold stronger affinity for D_2/D_1 receptor and similar D_2/D_1 selectivity ratio with Clozapine). Clozapine-like D_2/D_1 receptor occupancy profile was proved by *in vivo* evaluation. In addition, the reactive metabolites derived agranulocytosis risk of compound **5** was considered to be lower than Clozapine. The pharmacology detail of compound **5** is being investigated to develop it for TRS treatment.

Schizophrenia is a heterogeneous and severe neuropsychiatric disorder that affects nearly 1% of the population worldwide. Antipsychotic drugs are the mainstay of treatment, but 30% of patients are categorized as treatment-resistant schizophrenia (TRS).[1,2] TRS is defined as a non-response to at least two therapies of antipsychotic drugs. Clozapine is an effective medication for TRS with 60–70% of those treated showing a response, but its mechanism of action has not completely clarified.[3] Several hypotheses have been proposed. (1) Active metabolite: *N*-desmethylclozapine (NDMC) is a main metabolite of Clozapine, but plasma Clozapine/NDMC ratios were consistent in Clozapine-responders and non-responders. In addition, NDMC did not show a clear efficacy in a phase II clinical trial.[4] (2) Muscarinic receptor agonistic activity: Clozapine has an agonistic activity toward muscarinic M_1 receptor, while the structurally similar Olanzapine has no agonistic activity. M_1 agonistic activity of NDMC was stronger than Clozapine, but the clinical trial failed.[5] (3) Dopamine D_1 receptor antagonistic activity: it has been reported that D_1 receptor alleles predict the clinical response to Clozapine.[6] Selective D_1 antagonist, SCH-39116, showed efficacy in experimental animal models, but had no effect in a clinical trial.[7] Therefore, the development of Clozapine-like compounds have not been successful to date, since the mechanism of action of Clozapine was unclear (Fig. 1).

Patients with schizophrenia, when psychotic, show a heightened synthesis of dopamine and hyperdopaminergic state.[8] The increase in D_2 receptor stimulation is thought to be responsible of psychosis of schizophrenia and antipsychotic drugs help patients by occupying D_2 receptors, though too much D_2 antagonism induce side effects like extrapyramidal symptom and neuroleptic dysphoria.[9,10] Abi-dargham *et al.* reported that significant upregulation in D_1 receptor binding in the dorsolateral prefrontal cortex in patients with schizophrenia.[11] Mild blockade of D_1 receptor signaling would be needed as D_1 receptor signaling generally follows an inverted U-shape dose response curve.[12] Thus, moderate D_1 and D_2 antagonism would be the important key for adequate antipsychotic effect. It is noteworthy that the equivalent occupancy of striatal dopamine D_1 and D_2 receptors was demonstrated by Clozapine in a human PET study at the clinical dosing regimen (55% for D_1, 61% for D_2), whereas the occupation of other existing antipsychotic drugs did not show such behavior (Olanzapine, D_1: 43%, D_2: 79%; Quetiapine, D_1: 12%, D_2: 30%; Risperidone, D_1: 25%, D_2: 81%).[13] Similar clinical results were reported in other papers.[14,15] These studies suggest that this distinctive effect on D_1 and D_2 receptors may be responsible for the unique effectiveness of Clozapine in TRS. Recently, Lundbeck developed Zicronapine ($D_1 > D_2$ antagonist) for TRS, but the development of Zicronapine was terminated in a phase II clinical study. We consider that Zicronapine did not achieve an equivalent occupancy of D_2/D_1 receptor in its clinical study. In the current study, we tested the binding affinity of several compounds toward D_2/D_1 receptors, and the results are summarized in Table 1. D_2/D_1 receptor selectivity ratio of Clozapine was calculated to be 8.8, whereas there was a lower value for Olanzapine (1.2) and higher value for

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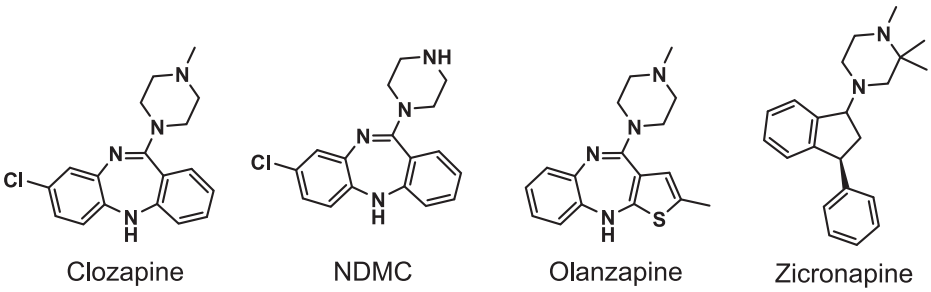


Fig. 1. Structures of antipsychotic drugs.

Table 1
SAR exploration of reference drugs and compounds **1–3.**

Compound	D_1 (ki; nM)	D_2 (ki; nM)	D_2/D_1 ratio	dGSH (mmol/L)
Clozapine	44	389	8.8	7.5
Olanzapine	47	58	1.2	0.077
Zicronapine	0.13	28	215	0
SCH39166	0.9	1,240	1,380	nt
1	380	1,380	3.6	0.21
2	55	1,180	21.4	0.99
(*S*)-3	51	479	9.4	1.3
(*R*)-3	780	> 1,400	–	0.84

nt; not tested.

Zicronapine (215). Based on the background clinical information, we speculate that Clozapine-like D_2/D_1 receptor selectivity ratio is the Clozapine-like D_2/D_1 receptor selectivity ratio is critical for demonstrating the efficacy in TRS.

Agranulocytosis is a life-threatening side effect of Clozapine, which limits the broader application of this highly effective drug.[16] The reactive metabolite formation has been proposed as a potential mechanism of the agranulocytosis caused by Clozapine, and was initiated by the activation of a nitrenium ion (illustrated in Fig. 2).[17,18] In order to identify the compounds that have Clozapine-like D_2/D_1 receptor selectivity ratio without agranulocytosis risk, we designed carbon atom-linked Clozapine analogues, *i.e.* piperazine was replaced with a piperidine ring to avoid nitrenium ion generation. Dansylated glutathione (dGSH) was used as the trapping agent for the quantitative estimation of reactive metabolites. Test compound was incubated with dGSH and human hepatocyte microsome fraction, and the amount of test compound-dGSH conjugate was measured by LC/MS/MS analysis. As shown in Table 1, the dGSH-conjugate formation of Clozapine was much higher than that of Olanzapine, a clinically safer antipsychotic. The amount of conjugate in compounds **1–3** were lower than Clozapine (1/6–1/34), suggesting that our strategy was successful. In particular, (*S*)-enantiomer of **3** ((*S*)-**3**) showed a similar D_2/D_1 receptor selectivity ratio with Clozapine. Wood *et al.* reported a structure and activity relationship (SAR) information of a Clozapine analogue, in particular, focusing on the substituent at the 2nd and 8th positions, although the substitutes were limited to hydrogen and chlorine atoms.[19] Thus, we initiated an optimization study based on compound (*S*)-**3** to identify the Clozapine-like compound for clinical development.

Common intermediate (8,11-dichloro-5*H*-dibenzo[b,e][1,4]diazepine) was prepared according to the reported method (Scheme 1).[20] The halogenated nitrobenzene was substituted with anthranilic acid by heating in DMF in the presence of Cs_2CO_3, the iron-catalyzed hydrogenation of the nitro group, followed by reaction with WSC to produce a cyclized intermediate. The intermediate was treated with phosphorous oxychloride under reflux in toluene in the presence of *N,N*-dimetheylaniline to form a chloride intermediate. This intermediate was further converted to target compounds **1–3** by treatment with appropriate boronate reagents (Suzuki-coupling). We used chirally pure (*S*)- or (*R*)-1,6-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine for the synthesis of (*S*)-**3** or (*R*)-**3**. Compounds **4–20** were (*S*)-enatiomers and prepared by a similar method to that of compounds **1–3** using appropriate substituted-anthranilic acid and halogenated nitrobenzene.

Firstly, we introduced various substituents at the 2nd position (R^2) of compound (*S*)-**3**. The results are summarized in Table 2. The

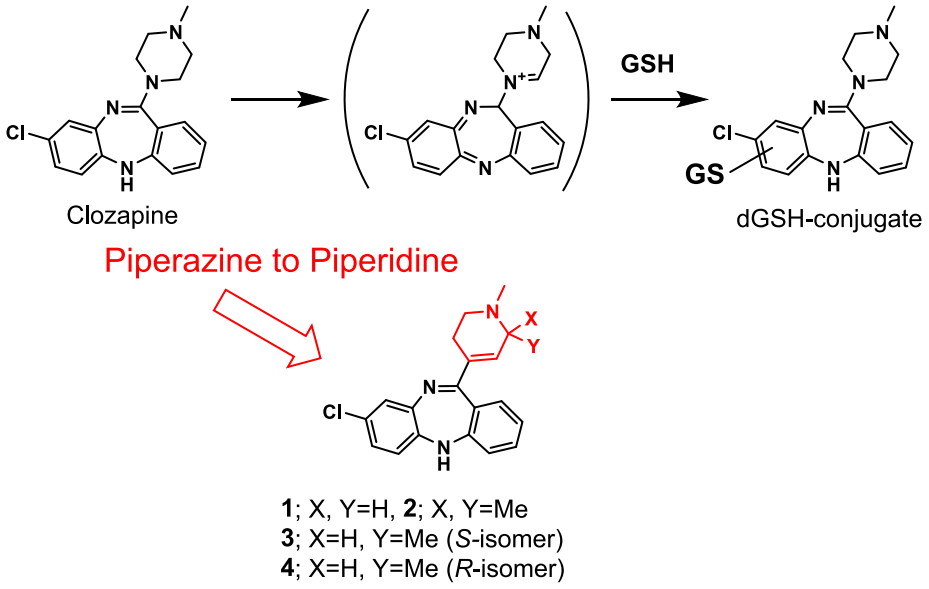
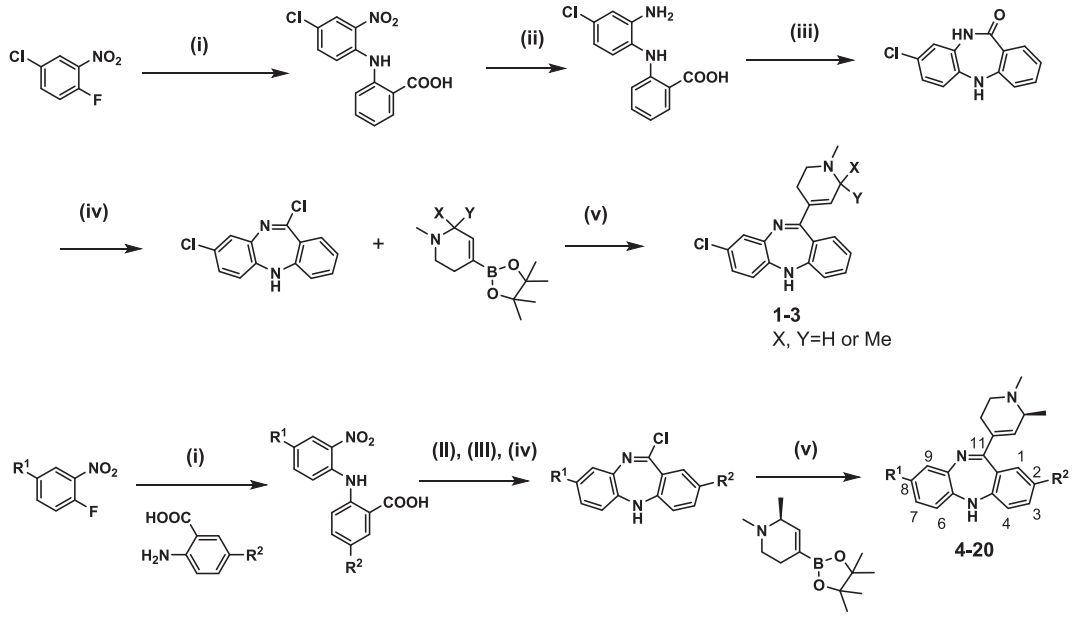
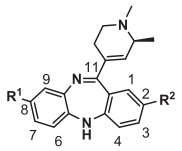


Fig. 2. Proposed route of Clozapine-GSH conjugation formation and our designed compounds **1–3.**



Scheme 1. Synthetic route for compounds **1–20** Reagents and conditions: (i) anthranilic acid, Cs_2CO_3, DMF, 120 °C. (ii) Fe, NH_4Cl, EtOH, reflux. (iii) HOBt, WSC, DMF, rt. (iv) $POCl_3$, *N,N*-dimethylaniline, toluene, reflux. (v) $Pd(PPh_3)_4$, Na_2CO_3, THF, reflux.

Table 2
SAR exploration of compounds **3–20.**



Compound	R^1	R^2	*Ki* (nM)		D_2/D_1 ratio	$5HT_{2A}$ (Ki; nM)	hERG (IC_{50}; μM)	dGSH (μmol/L)
			D_1	D_2				
Clozapine			44	389	8.8	1.4	3.2	7.5
Olanzapine			47	*58*	1.2	0.42	nt	0.077
***(S)*-3**	Cl	H	51	*479*	9.4	nt	nt	1.3
4	Cl	Me	2.3	63	28	0.094	0.8	0.35
5	Cl	Et	1.4	28	20	0.14	1.1	0.53
6	Cl	OMe	2	69	*35*	nt	*1.5*	0.89
7	Cl	OEt	3.7	*47*	13	0.091	0.9	0.77
8	Cl	OCF_3	2	110	*55*	0.14	1.9	0.58
9	Cl	F	0.2	127	*635*	nt	1.2	0.24
10	Cl	Cl	0.9	106	118	nt	0.9	0.72
11	Cl	CN	1.1	135	123	nt	0.6	0.49
12	Me	H	nt	> 1,400	–	nt	nt	nt
13	Me	Me	nt	> 1,400	–	nt	nt	nt
14	Me	OMe	nt	> 1,400	–	nt	nt	nt
15	Me	OCF_3	2.4	1218	508	nt	nt	nt
16	Me	F	*5.5*	453	82	nt	1.6	0.25
17	Me	Cl	0.48	98	204	nt	1.1	0.38
18	H	Me	7.9	819	104	nt	nt	0.85
19	H	Et	4	167.5	42	nt	0.6	0.86
20	H	Cl	1.6	124	78	nt	0.6	0.67

nt; not tested.

introduction of the substituent was efficacious in significantly improving the affinity toward the D_1 receptor, regardless of the electronic/steric nature of the substituents. The electron-donating groups (**4–8**) showed slightly stronger affinity on the D_2 receptor than the electron-withdrawing groups (**9–11**), such that the D_2/D_1 receptor selectivity ratios of compounds **9–11** were much higher than that of Clozapine. Among the electron-donating groups, compounds **5** and **7** appeared to be preferable profiles in terms of the D_2/D_1 receptor affinity and selectivity ratio. Next, the chlorine atom at the 8th position (R^1) of (*S*)-**3** was replaced with a methyl group. The molecular size of the methyl group was similar to that of the chlorine atom, but the electronic behavior was opposite, so we prepared and examined the D_1/D_2 receptor affinity of compounds **12–17**. As shown in Table 1, the affinity on the D_2 receptor showed an opposite trend to the 8-Cl analogues, *i.e.* the electron-donating groups (**13–15**) resulted in a loss or decreased the affinity toward the D_2 receptor. Interestingly, the affinity for the D_2 receptor was still demonstrated in medium molecular size electron-withdrawing groups (**17**). These results suggested that the electron-rich benzene ring did not fit well with the D_2 receptor. In the cases of hydrogen atom as the R^1 substituent (**18–20**), similar trends were seen with those of the 8-Me analogues. The results of compounds (*S*)-**3** and **20** showed similar trends with those of Clozapine *versus* Isoclozapine.[18] Among the compounds prepared, both compounds **5** and **7** displayed similar D_2/D_1 receptor selectivity ratio with Clozapine, and the potency was approximately 10-fold stronger than that of Clozapine. In CHO cells stably expressing human D_1 or D_2 receptor, both compounds **5** and **7** antagonized a dopamine-stimulated Ca^{2+} accumulation as well as Clozapine. Clozapine is categorized as a serotonine-dopamine antagonist, and $5HT_{2A}$ antagonism is also an important mechanism of action; Clozapine, compound **5** and **7** showed strong affinity for the $5HT_{2A}$ receptor, and *Ki* values were 1.3 nM, 0.094 nM and 0.091 nM, respectively. These results indicated that the potency of compound **5,** as well as the affinity for dopamine D_2/D_1 receptor, was stronger than that of Clozapine by over 10-fold.

As shown in Table 2, the amounts of dGSH-conjugation of all test compounds including compound **5** were lower than that of Clozapine by about 1/10-fold. This result suggested that our compounds may be considered to be low risk in term of the agranulocytosis derived from reactive metabolites. In addition, hERG/D_2 selectivity of compound **5** was larger than that of **7**; thus, compound **5** may be considered to be a safer compound from the viewpoint of cardiovascular toxicity, and we selected compound **5** for future evaluation.

Compound **5**, Clozapine, Olanzapine, and Zicronapine were selected for *in vivo* D_2/D_1 receptor occupancy (RO) evaluation. The test compounds were subcutaneously administered to male rats 1 h before racropride (a D_2 antagonist) or SCH-23390 (a D_1 antagonist) administration. D_1 or D_2 receptor occupancy in the brain striatum was evaluated by measuring racropride or SCH-23390 concentration by LC/MS/MS analysis. The test compounds bound the D_1 and D_2 receptor

Table 3
D_2/D_1 receptor occupancy profiles of reference drugs and compound **5**.

Compound	RO_{50} plasma free conc. (nM)		D_2/D_1 ratio RO_{50}	D_2/D_1 ratio (in vitro)
	D_1	D_2		
Clozapine	73.4	24.2	0.33	8.8
Olanzapine	480	3.8	0.0079	1.2
Zicronapine	6.6	23	3.5	215
5	6	1	0.17	20

dose-dependently, and the corresponding free plasma concentrations of RO_{50} and the D_2/D_1 selectivity ratio are summarized in Table 3. In the case of Clozapine, RO_{50} values of D_2 and D_1 were 24.2 and 73.4 nM, respectively, and the resulting D_2/D_1 selectivity ratio was calculated to be 0.33. Moreover, D_2/D_1 selectivity ratio of Olanzapine and Zicronapine were calculated to be 0.0079 and 3.5, respectively. The rank order of the ratio was well consistent with that of the in vitro results. The selectivity ratio of compound **5** was 0.17, which is close to that of Clozapine. These results indicated that the *in vivo* D_2/D_1 ratio of compound **5** was comparable to that of Clozapine, whereas those of Olanzapine and Zicronapine were not.

In conclusion, we investigated Clozapine-like compounds to develop for treatment of TRS based on dopamine D_2/D_1 receptor selectivity theory. The 11-tetrahydropiperidine analogue (*S*)-**3** displayed similar D_2/D_1 receptor selectivity ratio with Clozapine. We optimized the substituents at the 2nd and 8th positions of (*S*)-**3** and identified compound **5** to be the best compound (with approximately 10-fold stronger affinity for D_2/D_1 receptor and similar D_2/D_1 selectivity ratio with Clozapine). Clozapine-like D_2/D_1 receptor occupancy profile (RO_{50}) of compound **5** was validated by *in vivo* evaluation. In addition, the reactive metabolite-derived agranulocytosis risk of compound **5** was considered to be lower than that of Clozapine. Further biological investigation of compound **5** is underway to develop it for TRS treatment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127563.

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