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Viewpoint

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Classics in Chemical Neuroscience: Clozapine

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ABSTRACT: Clozapine was the first true breakthrough in schizophrenia treatment since the discovery of chlorpromazine in 1950, effectively treating positive, negative, and some cognitive symptoms, as well as possessing unprecedented efficacy in treatment-resistant patients. Despite over 30 years of intense study, the precise molecular underpinnings that account for clozapine's unique efficacy remain elusive. In this Viewpoint, we will showcase the history and importance of clozapine to neuroscience in general, as well as for the treatment of schizophrenia, and review the synthesis, pharmacology, drug metabolism, and adverse events of clozapine.

KEYWORDS: *clozapine, N-desmethylclozapine, schizophrenia, pharmacology*

Schizophrenia is a complex, heterogeneous neuropsychiatric disorder composed of positive, negative and cognitive symptom domains with a prevalence of approximately 1% worldwide.[1−6] Prior to the 1950s, treatment for schizophrenic patients focused on institutionalization (typically with heavy sedation) or electroconvulsive therapy.[1] Major milestones in the pharmacotherapy of schizophrenia occurred first in the 1950s, with the discovery of chlorpromazine (**1**) and haloperidol (**2**), so-called typical or first generation antipsychotics (FGAs),[1−6] followed in the 1970s by the development of clozapine (**3**), the first atypical or second generation antipsychotic (SGAs),[7−14] an advance that regained momentum in 1989 following reintroduction of clozapine to the market by the United States Food and Drug Administration (FDA) (Figure 1).[9,12−14] Chlorpromazine (**1**) and haloperidol (**2**), both potent D_2 antagonists, led to the development of the dopamine hypothesis of schizophrenia.[6,7,15,16] This model proposes that imbalances in dopamine transmission induce the presentation of particular symptomatic domains (i.e., hyperactivity in subcortical mesolimbic projections leads to positive symptoms and hypoactivity in mesocortical projections to the prefrontal cortex leads to negative symptoms and cognitive impairment).[6,7,15,16] Early data correlating clinical efficacy with D_2 potency provided credence to this hypothesis. However, the FGAs led to extrapyramidal side effects (EPS) via their actions at D_2 (muscle rigidity, tremors, Parkinsonian-like symptoms), which resulted in poor patient compliance.[6,7,15,16] Clozapine (**3**) was the first antipsychotic agent that dissociated clinical efficacy for positive symptoms from the risk of developing EPS, and 42 years later clozapine remains the one of the most clinically effective antipsychotics available, despite the development of numerous, structurally related SGAs and even third generation antipsychotics (TGAs).[1,7−14] Imaging studies suggest that while FGAs require striatal D_2 occupancy of ∼75% for efficacy (and EPS occur at ∼80% occupancy), SGAs, such as **3**, display <60% occupancy of striatal D_2 receptors.[13,14,17,18] Thus, D_2 inhibition alone cannot account for the efficacy of **3**. As will be detailed later in this Viewpoint, **3** is a preeminent example of serendipitous polypharmacology. In addition to D_2, clozapine has high affinity for the serotonin 5-HT_{2A} receptors, which led Janssen and Meltzer to propose a "dopamine-serotonin antagonism theory" wherein a high ratio of 5-HT_{2A} inhibition to D_2 inhibition accounts for the efficacy of atypical antipsychotics.[19,20] More recently the so-called *N*-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis has been advanced, which invokes circuit level dysfunction, particularly in glutamatergic circuitry, as a complementary hypothesis to describe the etiology of schizophrenia.[21−25] Clozapine's efficacy can be encapsulated under this hypothesis as well. Clozapine is a modest inhibitor of SNAT2, which increases synaptic glycine levels, thus activating NMDA receptors.[26] In addition, the major circulating metabolite of **3**, *N*-desmethylclozapine or NDMC (**4**), is an M_1 allosteric agonist, which has been shown to potentiate NMDA receptor currents.[27−29] Finally, there is also the cholinergic hypothesis of schizophrenia, where muscarinic agonism is desirable, and could potentially account for the improved efficacy of **3** and **4** on negative and cognitive symptoms in addition to the positive symptoms.[30−32] Recent positive schizophrenia trial data with xanomeline, an M_1/M_4 preferring agonist, further adds credence to the therapeutic role of **4** in the overall effectiveness of **3**.[33] Thus, the observed clinical efficacy of clozapine can be accounted for under any and/or all of the prevailing hypotheses used to explain the etiology of schizophrenia.

However, clozapine is not a panacea. Indeed, clozapine was once pulled from the market due to the risk of severe adverse events, only to be later introduced, as it was shown to be the most effective agent available for therapy in treatment-resistant schizophrenics.[7−14] Interestingly, it later also received labeling for effectively reducing recurrent suicidal behavior in patients with schizophrenia.[12−14,34] Patients must be now carefully monitored and the dose of clozapine must be titrated to avoid potentially fatal toxicity.[12−14,35] Thus, there is still an urgent

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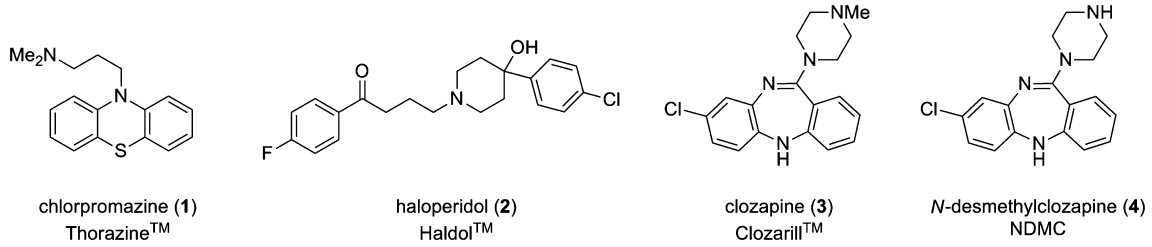


Figure 1. Breakthrough medications for schizophrenia. Structures of the FGAs chlorpromazine (**1**) and haloperidol (**2**), and the SGA clozapine (**3**) and its major active metabolite *N*-desmethylclozapine (**4**).

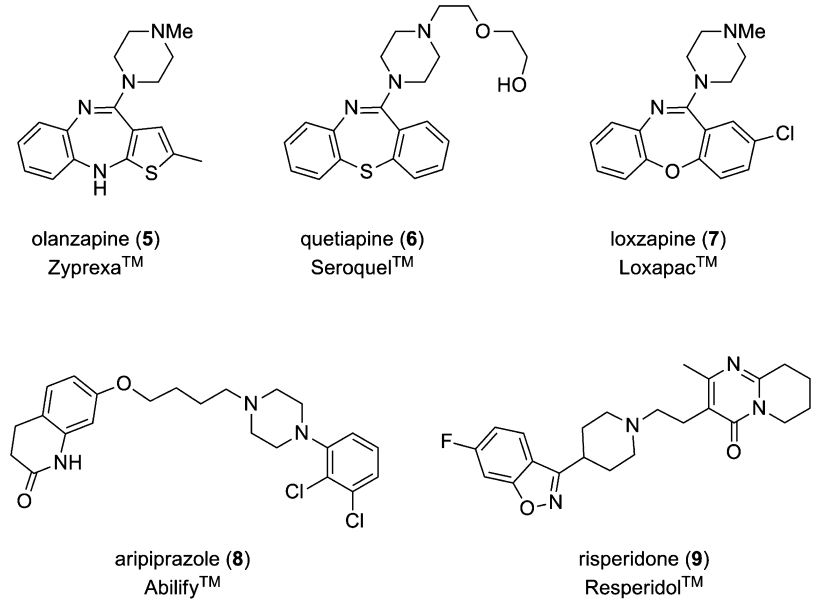
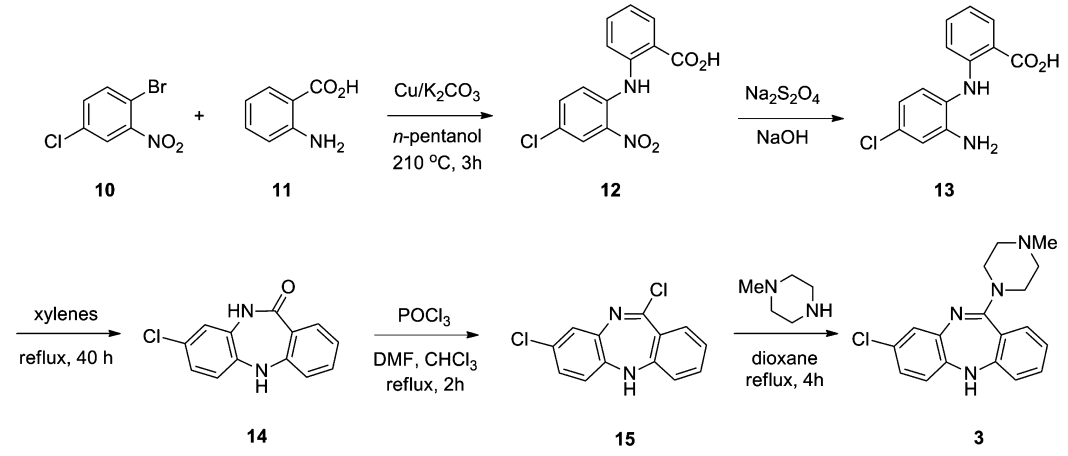


Figure 2. Structures of closely related SGAs **5**–**7** to clozapine and newer TGAs **8** and **9**.

Scheme 1. Synthesis of Clozapine 3 Reported in Both the Primary and Patent Literature



and unmet medical need for more effective antipsychotic agents, and despite the structural similarity to **3** other SGAs such as olanzapine (**5**), quetiapine (**6**), and loxapine (**7**) have not proven to be as effective, nor have TGAs such as aripiprazole (**8**) and risperidone (**9**) (Figure 2).[7−14]

In this Viewpoint, we will review the synthesis, pharmacology, drug metabolism, and adverse events of **3**. We will also showcase the history and importance of **3** to neuroscience in general, as well as for the treatment of schizophrenia. While there are several excellent reviews on certain aspects of clozapine,[12−14] the aim of this Viewpoint was to capture all the relevant data and compile it in an easily accessible format.

■ CHEMICAL SYNTHESIS

Clozapine (CAS No. [5786-21-0]) is a low molecular weight tricyclic benzodiazepine (MW = 326.13) with a lone hydrogen bond donor, three hydrogen bond acceptors, and a cLogP of 3.7. Thus, clozapine conforms to Lipinski's rules and displays excellent DMPK parameters and CNS penetration (vide infra). Originally developed by Sandoz (now Novartis), several

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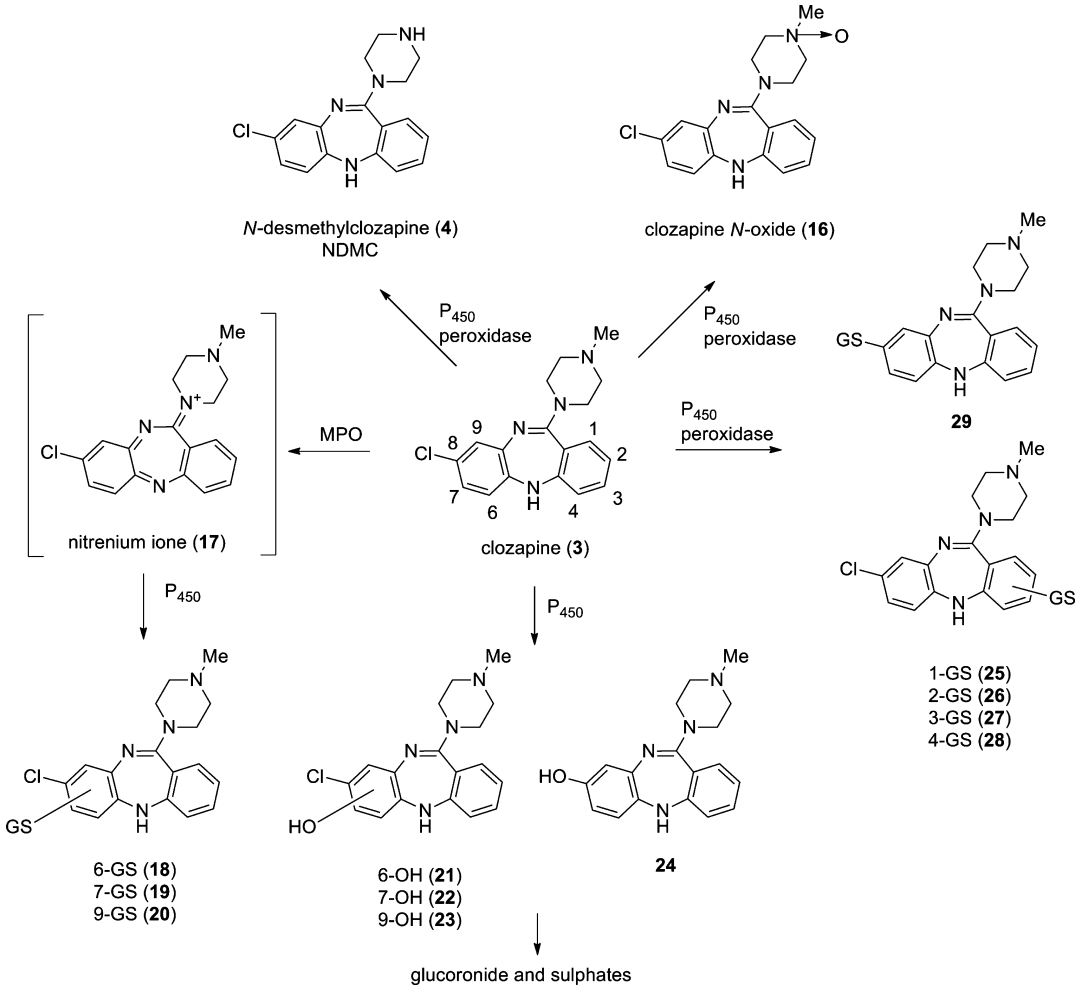

Figure 3. Structures of oxidative metabolites of clozapine **3**, and subsequent conjugative phase II metabolites.[42−45] The *N*-desmethyl metabolite **4** and the *N*-oxide **16** are the major metabolites, with **4** being active.

variants of the synthesis of the benzodiazepine-based clozapine (8-chloro-11-(4-methylpiperazin-1-yl)-5*H*-benzo[*b*,*e*][1,4]-diazepine, **3**) were reported in late 1960s, and in the issued patent U.S. 3,539,573 published in 1970.[36,37] These routes, and subsequent routes developed for analogue synthesis, first accessed the key 8-chloro-5*H*-benzo[*b*,*e*][1,4]diazepin-11-(10*H*)-one core **14** (Scheme 1). In the original reports, halogenated nitro arene **10** is subjected to an Ullmann coupling the presence of 2-aminobenzoic acid **11** to afford disubstituted aniline **12**. Reduction of the nitro moiety followed by refluxing in xylenes generates the key benzodiazepine core **14**. Treatment with $POCl_3$ provides the chloro imine derivative **15**, which is then treated with *N*-methylpiperazine to deliver clozapine **3**.[36,37] Other variations have been reported.[38,39] For example, the original patent also describes coupling *N*-methylpiperazine to **13** to generate the corresponding amide prior to thermal condensation leading to **3** directly. En route to a tritiated version of **3**, de Paulis and co-workers[39] first converted **14** to the thioamide, followed by methylation to provide an alternate leaving group for displacement by the tritiated *N*-methylpiperazine. In general, all the chemistries reported to access **3** and related analogues follow very similar synthetic routes.

MANUFACTURING INFORMATION

Clozapine is the generic name of the drug **3**, which is manufactured by Novartis (formerly Sandoz) under brand name Clozaril (other brand names employed for clozapine: FazaClo, Clopine, CloZAPine Synthon, Denzapine, Zaponex).[40,41] Clozapine was first synthesized in the 1960s, launched in Europe in 1971, voluntarily withdrawn in 1975, and reapproved by the FDA in 1989. Novartis sells clozapine in 25 mg and 100 mg tablets. There are also three generic manufacturers of 25 mg and 100 mg bioequivalent clozapine: Caraco (approved 2002), Teva (approved 1997), and Mylan (approved 1999). While sales figures for clozapine are difficult to ascertain precisely, worldwide sales are estimated to exceed U.S. $200 million.[12−14,40,41]

DRUG METABOLISM

Clozapine is a well-distributed CNS-penetrant compound that is 90−95% absorbed when administered orally without food).[12−14,40,41] However, due to a high first pass effect, the absolute oral bioavailability is only moderate (F = 0.5−0.6). Clozapine displays low binding to plasma proteins (F_u = 0.05) and peak plasma levels are achieved about2 h after oral dosing. The elimination of clozapine is biphasic, with a terminal half-life of approximately 12 h after steady state has been achieved (typically 7 days of dosing). Moreover, clozapine is extensively

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metabolized by CYP_{450} enzymes (especially 1A2 and 3A4), resulting in 80% of the dose excreted as metabolites in the urine (50%) and feces (30%).[12−14,40,41] These metabolites, **4** and **16**−**29**, have been rigorously characterized (Figure 3).[42−45] The vast majority of the metabolites are inactive, but the *N*-desmethyl metabolite **4**, NDMC, is active (vide infra) and can account for 10−90% of the circulating dose of administered clozapine.[12−14,28,28,46−49] The role of 1A2 in the metabolism of clozapine has been well characterized.[12−14] Agents that induce 1A2, such as cigarette smoke, increase the rate of clozapine's metabolism. Given the high incidence of cigarette smoking in the schizophrenic patient population, this can have a significant impact on medication management; smokers require increased dosage to maintain necessary plasma concentrations. Conversely, agents that inhibit 1A2, such as ciprofloxacin, decrease the metabolism of clozapine.[12−14,42−45] Overall, the ratio of **3** to **4** varies across patients, with female and elderly populations exhibiting the greatest variability. The impact of the ratio of **3**:**4** on efficacy will be discussed in the pharmacology section, but careful monitoring of plasma levels of both **3** and **4** are used to assess metabolism, compliance, and to adjust dosage.[12−14,40−49] In addition, the major toxicity that initiated the recall in 1975, agranulocytosis (vide infra), has been attributed to a reactive nitrenium ion **17** generated in neutrophils by myeloperoxidase (MPO) that leads to covalent modification.[42−44]

PHARMACOLOGY, ADVERSE EVENTS, AND DOSAGE

Without question, clozapine is the preeminent example of polypharmacology; more positively stated, clozapine is a broad spectrum ligand (Table 1).[12−14] Because **4** is an active metabolite, the mode of action becomes further complicated in vivo. As such, there is a great deal of controversy, regarding clozapine's unique efficacy and profile.[12−14] While clozapine is a D_2 antagonist, at therapeutic concentrations it occupies only 40−60% of D_2 receptors; in contrast, FGAs occupy >80% of D_2 receptors, which may account for the lack of extrapyramidal side effects with **3**.[13,14,17,18] This mild D_2 activity coupled with a broad spectrum of cholinergic, adrenergic, and serotonergic activity may give rise to the unique efficacy profile. Others argue that the preference for either D_1 or D_4 inhibition over D_2 is essential.[50] In addition to D_2, clozapine has high affinity for the serotonin 5-HT_{2A} receptors.[51] Beyond modulation of specific receptors, clozapine (**3**) and NDMC (**4**) have significant effects on GABA-ergic and glutamatergic circuitry.[12−14,52] Clozapine is a modest inhibitor of SNAT2, which increases synaptic glycine levels, thus activating NMDA receptors.[26] In addition, the major circulating metabolite of **3**, *N*-desmethylclozapine or NDMC, (**4**), is an M_1 allosteric partial agonist (EC_{50} = 115 nM), which has been shown to potentiate NMDA receptor currents.[28−32] The efficacy of clozapine can be then partially attributed to **4**, which also possess D_2 and 5-HT_{2A} activity comparable to **3** (Table 1),[53] and it is likely this combination of activity through modulation of glutamatergic and muscarinic neurotransmission, as well as numerous biogenic amines, that sets clozapine apart as a clinically effective treatment for treatment-resistant schizophrenia.[12−14,28−32] Intriguingly, a recent study raised concern over the classification of **4** as an M_1 agonist. In human brain tissue from post-mortem schizophrenic patients, a GTPγS assay showed that **4** was an M_1 antagonist.[54] However, we and others have recently shown that M_1 partial agonists display brain-region specific pharmacology (agonism/antagonism) based on receptor reserve.[55−57] GTPγS assays have no receptor reserve, and under these conditions partial agonists would behave as antagonists; thus, this potentially contradictory result can be explained. The role of **4** in the efficacy of clozapine therapy has been addressed in a clinical guideline, calling for consideration of the ratio of **3**:**4**.[12−14,28−32] While numerous accounts over the past 30 years have reported pharmacologic data for **3** and **4**, variability in assays and cell lines preclude a true head-to-head comparison; therefore, we elected to show data (Table 1) from the NIMH Psychoactive Drug Screening Program where cell lines and assay protocols are consistent.[53]

Table 1. Pharmacological Profiles of Clozapine (3) and *N*-Desmethylclozapine (4)[53a]

	K_i (nM)	
protein target	clozapine (**3**)	*N*-desmethylclozapine (**4**)
5-HT_{1A}	105	14
5-HT_{1B}	398	407
5-HT_{1D}	2100	476
5-HT_{1E}	966	393
5-HT_{2A}	13	11
5-HT_{2B}	7.5	2.8
5-HT_{2C}	29	12
5-HT_3	241	272
5-HT_5	3.8	351
5-HT_6	17	12
5-HT_7	18	60
α_{1A}	1.6	105
α_{1B}	7.0	85
α_{2C}	142	118
β_1	>10 000	6200
β_2	>10 000	4700
M_1	14	68
M_2	204	416
M_3	25	96
M_4	29	170
M_5	94	35
D_1	189	14
D_2	431	115
D_3	646	234
D_4	39	102
D_5	235	284
H_1	2.0	3.4
H_2	153	345
δ-opioid	>10 000	128
I_1	>10 000	758
SERT	1600	316
NET	3200	494

[a] K_i values as determined by the NIMH Psychoactive Drug Screening Program. http://pdsp.med.unc.edu/pdsp.php (accessed May 22, 2013).

A number of adverse events, ranging from relatively minor to fatal, have been reported with clozapine.[12−14,40,41,58,59] Among the most serious of these is agranulocytosis (vide infra), which led to the recall of clozapine in 1975. Overall, clozapine has five black box warnings (agranulocytosis, seizures, myocarditis, other adverse cardiovascular and respiratory effects, and increased mortality in elderly patients with dementia-related psychosis). Beyond this, hypotension, tachycardia, sedation/drowsiness, vertigo, bone marrow suppression, and rebound psychosis have been reported.[12−14,40,41,58,59] Another serious issue with clozapine is weight gain (typically a ≥7% increase in

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body weight over the first 6 months of therapy), which places patients at increased risk for diabetes and cardiovascular disease. Studies have shown that clozapine disrupts metabolism such that the body derives increased energy from fat versus carbohydrates, resulting in high carbohydrate levels that can ultimately lead to insulin resistance and diabetes. Other adverse events include urinary incontinence, withdrawal effects, hypersalivation ("wet pillow syndrome"), gastrointestinal hypomotility (severe constipation), and elevation of liver enzymes (typically around 10%). However, unlike the FGAs, clozapine has a low incidence of EPS and no effect on prolactin levels.[12−14,40,41,58,59]

Due to the risk of agranulocytosis, patients must have a blood test prior the initiation of clozapine therapy, and must have their white blood cell counts (WBC) and absolute neutrophil counts (ANC) monitored during their course of treatment and for 4 weeks after treatment ends.[12−14,40,41,58,59] To qualify for clozapine treatment, counts must be within normal range ((WBC $\geq$ 3500/mm^3 (3.5 $\times$ 10^9/L) and ANC $\geq$ 2000/mm^3 (2.0 $\times$ 10^9/L)). Qualifying patients initiate therapy with one to two 12.5 mg doses orally on day one, followed by one to two 25 mg oral doses on day two. If tolerated, the dose can be escalated over 2−3 weeks in 25 to 50 mg increments until a 300 mg/day dose is achieved. For the majority of patients, antipsychotic efficacy is noted between 200 to 450 mg/day, typically divided unequally with the largest portion being administered at bedtime. If required, the dose can be further increased in increments of 50 to 100 mg. The maximum dose allowed is 900 mg/day, but adverse events grow increasingly common at doses exceeding 450 mg/day. When clozapine therapy is ended, a gradual reduction in dose over a one to two week period is recommended, as abrupt cessation can lead to withdrawal symptoms.[12−14,40,41,58,59]

■ HISTORY AND IMPORTANCE IN NEUROSCIENCE

Imagine a time when the standard of care for patients with schizophrenia is institutionalization (typically with heavy sedation) or electroconvulsive therapy.[1] Then, in the 1950s, small molecule therapeutics appear, initially as a panacea, with chlorpromazine (**1**) and haloperidol (**2**) reducing psychotic (or positive symptoms) of schizophrenia. However, these FGAs induce EPS and Parkisonsonian-like motor disturbances, due to their potent D_2 inhibition.[1−6] Still, their introduction is a landmark in schizophrenia care, despite the side effect profiles and lack of efficacy on the negative symptoms and cognitive deficits, which are key determinants of long-term disability and treatment outcome.[1−6] Furthermore, 30−70% of schizophrenic patients are nonresponsive to these agents for remission of positive symptoms, and are considered to have treatment-resistant schizophrenia (schizophrenia patients that despite at least two adequate trials of standard neuroleptic drugs, have persistent moderate-to-severe positive symptoms, disorganization or negative symptoms, together with poor social and work function over a prolonged period of time).[1−14]

Clozapine (**3**) enters the scene in Europe in 1971 as the first agent to dissociate antipsychotic efficacy from EPS and other motor side effects, a major landmark in the treatment of schizophrenia that paves the way for other SGAs and TGAs.[1−14] Moreover, clozapine is eventually found to be effective on negative symptoms as well as improving certain domains of cognitive function (memory, verbal learning, verbal fluency, and psychomotor speed) but not others (executive function and working memory). Needless to say, psychiatrists are thrilled with the prospects of clozapine in managing the multisymptom domains of their schizophrenic patients. The momentum comes to a screeching halt in 1975 based on reports from Finland, where 16 patients out of 2206 (0.7%) developed agranulocytoisis, an acute condition resulting in lowered white blood cell count(commonly neutrophils), placing patients at high risk of infections due to suppressed immune systems. Of the 16 patients, 8 (50%) developed secondary infections and died; however, no other clustering of agranulocytoisis was reported in Finland or in any other countries. Still, Sandoz voluntarily withdrew clozapine from the market in Europe and ongoing clinical trials elsewhere in the world were suspended.[1−14]

During the ensuing years after the withdrawal, many European patients that had responded well to clozapine relapse failed to respond to any of the available antipsychotic drugs. Thus, under pressure from this patient group, administration of clozapine is permitted on a restricted basis. Studies then show that clozapine is effective in treatment-resistant schizophrenia, improving positive symptoms as well as tardive dyskinesia (a motor disorder resulting in involuntary, repetitive body movements), again distinguishing itself from classical FGAs and newer SGAs.[1−14] Due to a lack of new, effective agents, in 1989, Sandoz seeks approval from the FDA for clozapine in patients with treatment-resistant schizophrenia, and wins approval. All regulatory agencies worldwide, including the FDA, require blood testing for patients taking clozapine, to monitor for the development of agranulocytoisis. Typically, the risk of agranulocytoisis decreases 10-fold over the first six months, and with monitoring, incidence has now dropped to 0.38%. Multiple trials validate the efficacy of clozapine on treatment resistant schizophrenics, with around 30% showing improved Brief Psychiatric Rating Scale scores after 6 weeks of treatment, and nearly 70% show improvement after 6 months of treatment.[1−14] Later, clozapine also shows efficacy in adolescents with treatment-resistant schizophrenia. Comparable efficacy is noted for the treatment negative symptoms, though the impact on cognitive deficits is not as clear (possibly owing to the variability in the metabolism of **3** and concentration of **4**).[1−14]

Over the intervening years, it is recognized that yet another unmet medical need in this patient population is an elevated mortality rate. Recent data indicate that people with schizophrenia have a higher incidence of suicide, with a lifetime attempt rate of about 60% and a completed suicide rate of 9−13%.[1−14] Suicide rates are seen to be higher in males and in the early stages of the disease. In several trials, clozapine is shown to significantly reduce the rate of suicide attempts by as much as 88% when compared to FGAs and other SGAs. A retrospective analysis of the 100 000 patient Clozaril trial reveals a suicide rate 25−50% lower than expected.[1−14] These data led the FDA to approve clozapine in 2002 for reducing the risk of recurrent suicidal behavior in patients with schizophrenia. Overall, clozapine is shown to offer numerous advantages over other SGAs and TGAs even today (Table 2).[1−14]

A final issue often associated with clozapine therapy centers on the cost-effectiveness, for which there have been numerous studies and with diverse outcomes.[12,14] Estimated costs for a typical patient (300−400 mg/day of clozapine) coupled with blood monitoring is ∼$5500/year, a figure almost 11-times greater than other conventional SGAs.[12,14,60] However, some feel that the added expense of clozapine treatment is offset by

Table 2

clinical advantages of clozapine (3)
robust efficacy in treatment-resistant patients
improved coutcome for partial responders (vs standard FGAs/SGAs)
robust efficacy on positive symptoms
robust efficacy on negative symptoms
improved disorganized behavior
improved some aspects of cognitive deficits
only FDA-approved agent to lower suicide risk
efficacy on depression
diminished aggressive behaviors
no extrapyramidal symptoms (EPS)
no tardive dyskinesia
no increase in serum prolactin
improved compliance

the corresponding reduction in hospital costs and crisis care, often required for aggressive and/or treatment-resistant schizophrenics. Indeed, Meltzer found that clozapine therapy reduced direct costs associated with treatment-resistant schizophrenics by ∼50% per year.[15] Other studies find variable savings; however, it is hard to estimate the cost borne by families and caregivers, lost wages, and so forth. Clearly, the ability of treatment-resistant patients to return to productive lives and jobs, freeing family members to return to work, further factors in to the cost savings realized by clozapine treatment.[12,14,60]

In summary, despite turning 42 this year, clozapine remains one of the most uniformly effective antipsychotic agents available, despite its significant adverse event profile, including five black box warnings (worst side effect profile of any antipsychotic). Clozapine was the first true breakthrough in schizophrenia treatment since the discovery of chlorpromazine in 1950, and it effectively treats positive, negative, and some cognitive symptoms. Moreover, clozapine is effective in treatment-resistant patients and has been shown to reduce the risk of suicide. Despite over 30 years of intense study, the precise molecular underpinnings that account for clozapine's unique efficacy remain elusive. Perhaps the ability of clozapine and its major metabolite NDMC to modulate biogenic amines and complex glutamatergic, GABAergic, and cholingergic circuitry serendipitously strikes the right balance of activities needed to treat the diverse, heterogeneous patient population present in schizophrenia. Many clinicians feel that clozapine is under-prescribed, and it is estimated that only 14−50% of clozapine-eligible patients are on the medication. Furthermore, there is a clear decline in the use of clozapine, despite the overwhelming benefits for unmet medical need in this patient population, and this trend is thought to be driven by aggressive marketing of current SGAs and TGAs.[12,14,61] Unfortunately, this may lead to a new generation of clinicians unfamiliar with prescribing, managing, and monitoring clozapine, leading to less than optimal care for treatment-resistant schizophrenics that may result in more hospitalization.[14,61] For fundamentally changing the standard of care in patients with schizophrenia and remaining an important, frontline therapy for over 40 years, we firmly believe that clozapine represents a *classic* in chemical neuroscience.

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Notes

The authors declare no competing financial interest.

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