**PERPHENAZINE 4-AMINOBUTYRATE MESYLATE**

*Dopamine D₂ Receptor Antagonist  
GABA<sub>A</sub> Receptor Agonist  
Treatment of Schizophrenia*

**CYP-1020**  
BL-1020 (free base)  
AN-168 (hydrochloride)

4-Aminobutyric acid 2-[4-[3-(2-chloro-10H-phenothiazin-10-yl)propyl]piperidin-1-yl]ethyl ester (tris)methanesulfonate  
InChI: 1S/C25H33ClN4O2S.3CH3SO3H/c26-20-8-9-24-22(19-20)30(21-5-1-2-6-23(21)33-24)12-4-11-28-13-15-29(16-14-28)17-18-32-25(31)7-3-10-27;3*1-5(2,3)4/h1-2,5-6,8-9,19H,3-4,7,10-18,27H2;3*1H3,(H,2,3,4)

**SYNTHESIS***

N-Protection of 4-aminobutyric acid with Boc₂O gives N-Boc-4-aminobutyric acid (II) (I), which by esterification with perphenazine (III) by means of either CDI in DMF (1-4) or DCC and DMAP in CH₂Cl₂ (1, 3) affords the amino ester (IV). Alternatively, treatment of N-Boc-GABA (II) with t-BuCOCl in the presence of Et₃N in THF gives the mixed anhydride (V), which by reaction with perphenazine (III) in THF yields amino ester (IV) (3). Finally, compound (IV) is deprotected by means of MsOH in acetonitrile (1, 3), Scheme 1.

**SUMMARY**

Schizophrenia is a lifelong disorder characterized by bizarre delusions, lack of motivation, reduction in spontaneous speech, social withdrawal and some affective symptoms. Perphenazine 4-aminobutyrate mesylate (CYP-1020) is a novel, mutual prodrug ester of GABA and perphenazine that exhibits potent dopamine D₂ receptor antagonist and GABA<sub>A</sub> receptor agonist activity. In relevant animal models, it showed antipsychotic activity with minimal cataleptic manifestations, and an increase in dopamine efflux in both medial prefrontal cortex and hippocampus. CYP-1020 increases prolactin levels, and oral drug has been demonstrated to deliver GABA to the brain. In human studies, CYP-1020 elicited dose-dependent striatal dopamine D₂ receptor occupancy, and in a clinical trial doses of 20-30 mg/day significantly improved psychotic symptoms and cognition, and the agent was safe and well tolerated in patients with schizophrenia. CYP-1020 is currently in a phase Ib/II clinical trial to assess its cognitive and antipsychotic efficacy, safety and tolerability compared to risperidone in patients with schizophrenia.

**Key words**: Antipsychotic – Schizophrenia – Perphenazine 4-aminobutyrate mesylate – CYP-1020

**BACKGROUND**

Schizophrenia is a lifelong disorder whose main features are bizarre delusions, lack of motivation, reduction in spontaneous speech, social withdrawal and some affective symptoms (5). In fact, schizophrenia encompasses a range of positive, negative, cognitive, mood and motor symptoms, the severity of which varies across patients and through the course of the illness (6).
Positive symptoms that are reality distortions, such as delusions and hallucinations, and negative symptoms that represent a substantial reduction in motivation, drive and the capacity to feel pleasure (5, 6), are both usually assessed in a research context by the Positive and Negative Syndrome Scale (PANSS) score (7). Concerning the epidemiology of schizophrenia, 15.2 new cases per 100,000 persons per year are diagnosed, about 7 individuals per 1,000 will be affected during their lifetime and people with schizophrenia have a 2- to 3-fold increased risk of dying in comparison with the general population (8); thus, there is a gap of 12-15 years in mortality between schizophrenic patients and the average population that is increasing (9).

The dopaminergic hypothesis, which postulates an increased dopamine release or sensitization as one of the major causes of schizophrenic manifestations (10, 11), is consistent with the fact that all of the clinically effective antipsychotics block dopamine receptors (12). Neurochemical imaging studies have now demonstrated that schizophrenia is associated with an increase in dopamine synthesis, dopamine release and resting state synaptic dopamine concentrations (13).

The first generation of antipsychotic agents, called typical antipsychotics, e.g., haloperidol, chlorpromazine, perphenazine and fluphenazine, were potent blockers of the dopamine D_2 receptor, showing efficacy against positive symptoms, but often producing extrapyramidal signs and tardive dyskinesia (14).

The second generation of antipsychotic agents, atypical antipsychotics, include olanzapine, quetiapine, risperidone and clozapine, which exert some blocking effects on the D_2 receptor, but also show affinity for 5-HT and norepinephrine receptors (14). The second generation of antipsychotic agents are effective in treating positive symptoms, with a reduced incidence of motor side effects, but the intended efficacy against the negative and cognitive symptoms has not been confirmed. Additionally, they induce metabolic side effects, i.e., weight gain, increased triglycerides and cholesterol (15, 16).

The overall efficacy and tolerability of the currently available antipsychotic drugs are limited, as evidenced by the CATIE clinical trial results, which showed a 78% discontinuation rate for any antipsychotic medication within 18 months due to poor efficacy and side effects (17). However, two additional issues can explain this discontinuation: the stigma of being labeled as psychotic (18) and the dampening of motivational drives elicited by the dopamine-blocking medication (19).

Thus far, highly selective drugs have not met full success in treating schizophrenia. On the contrary, the more effective drugs are those...
acting on multiple receptors or processes (20). This is why, in the search for new antipsychotic agents, other receptors and processes different from dopamine have been explored. In fact, there is an association between γ-aminobutyric acid (GABA) deficit and decreased activity of the rate-limiting enzyme in the conversion of glutamate to GABA and schizophrenia (21-23). In addition, GABA improves the cognitive deficits of schizophrenia and attenuates the extrapyramidal symptoms related to dopamine blockade (23). Some studies suggest that drugs targeting GABA_A receptors could be useful in the treatment of schizophrenia (24).

One way to circumvent the fact that GABA does not cross the blood–brain barrier (BBB) could be administering GABA in the ester form, which would cross the BBB and be released in the brain by hydrolysis (1). Perphenazine 4-aminobutyrate mesylate (CYP-1020), a novel mutual prodrug ester of GABA and perphenazine that exhibits dopamine receptor antagonism and GABA_A receptor agonism, is currently in phase IIb/III clinical trials at BioLineRx to assess its cognitive and antipsychotic efficacy, safety and tolerability compared to risperidone in patients with schizophrenia (25). A review of the early studies of CYP-1020 has already been published (26).

**PRECLINICAL PHARMACOLOGY**

In a receptor ligand binding screening test, CYP-1020 interacted strongly with human dopamine D_2L and D_2S receptors (K_i = 0.066 and 0.062 nM, respectively), and remarkably with 5-HT_2A (K_i = 0.211 nM) and histamine H_1 receptors (K_i = 0.473 nM). In addition, it showed agonist activity for the GABA_A receptor but not for the metabotropic GABA_B receptor. Perphenazine compared with CYP-1020 showed 3- and 5-fold lower affinities for D_2L and 5-HT_2A receptors, respectively, and no agonist activity for the GABA_A receptor. CYP-1020 is metabolized to perphenazine and GABA, and therefore its overall activity in vivo will probably be a mixture of the three compounds (27).

The effects of CYP-1020 and perphenazine were studied in experimental in vivo models of schizophrenia. In the amphetamine-induced hyperactivity model in rats (28), CYP-1020 administered as a single oral dose significantly reduced hyperactivity, with an efficacy slightly lower than an equimolar dose of perphenazine, but, in contrast to perphenazine, without inducing catalepsy when assessed in the same animals (27). In another experiment in the same model, oral CYP-1020 (at an equimolar dose of perphenazine) reduced hyperactivity, with minimal sedation and without catalepsy; perphenazine at 2.5 mg/kg p.o. reduced hyperactivity but produced sedation and catalepsy, and GABA at 1 mg/kg p.o. was without effect on hyperactivity. In a separate experiment to assess the ability to induce catalepsy, rats were treated orally with CYP-1020 at equimolar doses of perphenazine administered at 5, 10 and 20 mg/kg. CYP-1020 showed lower cataleptic behavior and caused decreased sedation in comparison with perphenazine. Muscular rigidity was observed only in animals treated with perphenazine, while maximum catalepsy was reached for both compounds at 4-5 hours after treatment (1).

In the model of amphetamine-induced hyperlocomotion in rats assessing the movements by photocells (29), subchronic treatment with oral CYP-1020 for 17 days at 3 dose levels, caused a dose-dependent reduction in hyperlocomotion that was slightly less potent than that elicited by perphenazine at equimolar doses. In this experimental setting, CYP-1020 again showed significantly reduced cataleptic manifestations in comparison with perphenazine (1, 27, 30-32).

The effects of CYP-1020 on cortical and hippocampal dopamine and acetylcholine release were studied in rats by means of microdialysis technology. Subcutaneous CYP-1020 dose-dependently increased dopamine efflux in both medial prefrontal cortex and hippocampus, without affecting acetylcholine release in these areas (27). CYP-1020 resulted in increased dopamine efflux, which may be relevant in the experimental paradigm of the phencyclidine-induced deficit in object recognition memory in rats, because this deficit is associated with impaired dopamine release in the prefrontal cortex and hippocampus. CYP-1020 administered orally for 15 days attenuated the effects of phencyclidine, suggesting that CYP-1020 may be useful for treating cognitive deficits in schizophrenia (33).

When studying the effects of oral CYP-1020 on behavioral and physiological responses in rats (Irwin test) only slight sedation and abnormal gait were observed starting at the dose of 20 mg/kg (27). The effects of oral CYP-1020 and perphenazine on prolactin levels were tested in rats. Both compounds administered at equimolar doses produced similar and significant increases in plasma prolactin levels. Clinical trials will clarify the impact of this issue (1, 27, 30).

**PHARMACOKINETICS AND METABOLISM**

A pharmacokinetic study performed in rats treated with [14C]-CYP-1020 at 5 mg/kg p.o. showed peak levels of radioactivity at 1 hour, with a plasma C_max of 1.4 μg/mL and a terminal half-life of 8.55 hours. After 24 hours, 36% of the label was recovered in the urine, 19% in the feces and only 7% in the carcass, therefore indicating excellent bioavailability and extensive metabolism. Taking into account that the radioactive labeling is on the GABA moiety of CYP-1020, the aforementioned data can only reflect the kinetics of GABA, because esterases may split CYP-1020 into perphenazine and GABA (27).

In order to ascertain whether CYP-1020 crosses the BBB intact, and if its metabolites can be found in the central nervous system, [14C]-CYP-1020 was administered to rats at 5 mg/kg p.o., and brain tissue was obtained at different times post-administration. CYP-1020 peaks were observed in brain tissue at 15 and 30 minutes, being difficult to detect from 1 hour on. GABA peaks were observed in brain tissue at 15, 30 and 60 minutes and 4 hours after administration (27).

Striatal dopamine D_2 receptor occupancy in vivo, assessed by positron emission tomography (PET), has been demonstrated to be a reliable predictor of antipsychotic response and drug-induced side effects, including extrapyramidal symptoms, akathisia and prolactin elevation (34). Consequently, a clinical trial to assess the degree of D_2 receptor occupancy in the human brain was performed in healthy male volunteers for oral CYP-1020 at single doses of 16, 24 and 32 mg, in comparison with perphenazine 8 mg. In addition, blood samples were taken to determine plasma perphenazine, CYP-2010 and prolactin levels. CYP-1020 administered in the range of 16-32 mg as a single dose showed a dose-dependent striatal D_2 receptor occupancy, which reached a maximum of 44% at 4-6 hours after admin-
Perphenazine demonstrated a faster and more potent effect in increasing prolactin plasma levels than an equimolar dose of CYP-1020, also demonstrating a delayed profile for CYP-1020 in comparison with perphenazine, which may be due to a delayed absorption or to the rate of formation of perphenazine from CYP-1020 (36-39).

SAFETY

In the aforementioned striatal D₂ receptor occupancy study, safety was also evaluated and CYP-1020 appeared to be safe and well tolerated in the range of doses studied. The most frequently occurring adverse events were fatigue, polyuria and nasopharyngitis. There were no significant changes in ECG, vital signs, UKU Side Effect Rating Scale after treatment, nor on physical examination or laboratory values (36-39).

A single-dose-escalating, double-blind, placebo-controlled phase I study was performed with CYP-1020 to assess its safety and tolerability. Subjects were hospitalized for 24 hours and adverse events, vital signs and cardiac, psychological and neurological events were recorded. CYP-1020 was well tolerated at all dose levels. A dose-dependent elevation of serum prolactin was observed. One of 48 subjects showed transient facial twitching and sleepiness after the dose of 40 mg (40, 41).

The first study to assess the tolerability and safety of CYP-1020 in schizophrenia was performed in 36 psychotic patients (males and females aged 16-65 years) who were treated for 6 weeks with CYP-1020 in the dose range of 20-40 mg/day (42-44). This was an open-label, multicenter phase II clinical trial in hospitalized patients with chronic schizophrenia or schizoaffective disorder, diagnosed at least 1 year before screening, required to be on antipsychotic treatment and with the current episode present for at least 1 month. The diagnosis was performed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, and the symptomatic assessed according to the Positive and Negative Syndrome Scale (PANSS) and the Clinical Global Impression-Severity (CGI-S). Patients were hospitalized during the first 14 days of the study, but thereafter could be treated as outpatients based on the rating obtained following the Readiness for Discharge Questionnaire (RDQ). The patients were followed for 30 days after the last dose for the occurrence of any serious adverse events. CYP-1020 was administered once daily at the starting dose of 20 mg, which was increased to 30 mg on day 4 and to 40 mg on day 7 if tolerance allowed it. CYP-1020 tolerability and safety were based on the incidence of adverse events, abandonments due to adverse events, ratings on the Extrapyramidal Symptom Rating Scale (ESRS), vital signs, electrocardiogram, laboratory tests and physical examination. All patients tolerated the starting dose of 20 mg, and all but 1 were switched to the dose of 30 mg on day 4, and on day 7, 30 of the 35 patients had their dose increased to 40 mg. Only one patient discontinued treatment due to adverse events (moderate parkinsonism), and only one patient showed a serious adverse event (psychomotor agitation, insomnia, suicidal ideation). All adverse events were of either mild or moderate severity, except for a case of severe akathisia, the most common adverse event being insomnia (six patients). The changes in the ESRS score were generally small. No cardiovascular alterations were observed, nor changes in the ECG intervals, including QTc lengthening. Laboratory testing showed only a significant, dose-dependent increase in plasma prolactin. These data suggest that future studies will assess doses of 10-30 mg/day.

In a randomized, double-blind, placebo- and risperidone-controlled, 6-week phase II clinical trial (EAGLE; 45) that was performed to assess the efficacy of CYP-1020 in patients with schizophrenia, some additional measurements concerning safety were performed. CYP-1020 (20-30 mg/day) showed a maximum change from baseline in the ESRS score comparable to that of risperidone (2-8 mg/day), whereas the CYP-1020 increase in prolactin levels was significantly lower than that of risperidone. Finally, there were no changes in ECG, laboratory or vital signs (46-48).

CLINICAL STUDIES

Although the objective of the open-label, multicenter phase II clinical trial (42-44) was to assess the tolerability and safety of CYP-1020 in hospitalized patients with chronic schizophrenia or schizoaffective disorder, the agent showed significant improvements in PANSS, CGI-S and CGI-C scores. In addition, the rate of patients ready for discharge, based on the RDQ, increased from 34% on day 14 to 71% on day 42 of the study. These data, together with the low dropout rate, suggested that CYP-1020 may be an effective drug.

In the EAGLE study, the 363 patients enrolled were randomized to receive treatment with 10 mg/day or 20-30 mg/day of CYP-1020, risperidone at 6-8 mg/day or placebo. The efficacy was measured by means of the PANSS, the CGI-S and CGI-C scores. Additionally, the effects on cognition were assessed by the Brief Assessment of Cognition in Schizophrenia (BACS). According to the PANSS scores, the high dose of CYP-1020 and risperidone showed significant improvement with respect to placebo, and no difference was seen between CYP-1020 and risperidone. The number of responders with the high dose of CYP-1020 was significantly higher compared with placebo following CGI-S and CGI-C assessment. Finally, the effects on cognition elicited by CYP-1020 at the high dose, as scored by the BACS composite score, indicated significant superiority compared with placebo and risperidone at the end of the study. These results are consistent with the CYP-1020 profile as an antipsychotic agent with GABA activity and potential for improving cognition (46-48).
Currently, a phase Ib/III study of CYP-1020 is ongoing to assess the cognitive and antipsychotic efficacy, safety and tolerability of the agent compared to risperidone in patients with schizophrenia (25).

CONCLUSIONS

Schizophrenia is a lifelong disease characterized by bizarre delusions, lack of motivation, reduction in spontaneous speech, social withdrawal and some affective symptoms. Although the new antipsychotic agents are effective in treating symptoms with a reduced incidence of motor side effects, the overall efficacy and tolerability of the currently available antipsychotic drugs are limited.

CYP-1020 is a novel mutual prodrug ester of GABA and perphenazine that exhibits potent dopamine D_2 receptor antagonist and GABA_A receptor agonist activity. In relevant animal models of schizophrenia, CYP-1020 showed antipsychotic activity with minimal cataleptic manifestations, and a dose-dependent increase in dopamine efflux in both medial prefrontal cortex and hippocampus in rats, suggesting that it may be useful for the treatment of cognitive deficits in schizophrenia. Consistent with its antidopaminergic properties, CYP-1020 increases prolactin levels, and in radiolabeled pharmacokinetic studies, oral CYP-1020 was demonstrated to deliver GABA to the brain. In human studies, CYP-1020 elicited a dose-dependent striatal D_2 receptor occupancy, and in a phase II clinical trial, CYP-1020 at 20-30 mg/day significantly improved the PANSS score and cognition, and was safe and well tolerated. CYP-1020 is currently in a phase Ib/III clinical trial at BioLineRx to assess its cognitive and antipsychotic efficacy, safety and tolerability compared to risperidone in patients with schizophrenia.

SOURCE

BioLineRx, Inc. (IS).

DISCLOSURES

The author states no conflicts of interest.

REFERENCES


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