Research Paper

Synthesis and Evaluation of Clozapine and its Related Compounds

Nagaraju Garipelli1*, B Madhava Reddy1, Jithan AV2
1G Pulla Reddy College of Pharmacy, Hyderabad, AP, India
2Vaagdevi College of Pharmacy, Hanamkonda, Warangal, AP, India

ABSTRACT: Clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine) is a dibenzodiazepine derivative, used in the treatment of schizophrenia. The present work aimed to develop a new modified method for the synthesis of clozapine and also impurity profiling which is mandatory by the regulatory bodies. Present synthetic method for clozapine has the advantages over the previous processes in respect of safety, cost and efficiency. Impurity profiling indicated that 8-chloro-10,11-dihydro-11-oxo-5H-dibenzo[b,e][1,4]diazepine, clozapine dimer, desmethyl clozapine, and clozapine N-oxide are very minor impurities in the synthesis of Clozapine. These compounds were confirmed by analytical data. The impurities were also synthesized and evaluated for the neuropharmacological activity. The study revealed that three of four impurities have decreased the locomotor activity in mice, which indicates the similar activity compared to clozapine as antidepressant

KEYWORDS: Clozapine, impurities, synthesis, locomotor activity.

Introduction

Schizophrenia is a severe, idiopathic psychiatric disorder with a polygenic component. It is composed of severe thought disorders, termed psychoses, which are characterized by illogical, delusional, or paranoid thoughts (Jaine N Delgado et al., 1998). Schizophrenia is the fourth largest disease in the world in the age group of 15-30 and posing it as one of the major medical challenges. The medical management of schizophrenia often requires a combination of antipsychotic, antidepressant, and antianxiety drugs (Helio Elkis., 2007). Disruption of the dopamine system has been strongly linked to Schizophrenia and related psychosis, with abnormally high dopamine action apparently leading to these conditions. Numbers of drugs having the activity for blocking the neurotransmission of dopaminergic receptor in the central nervous system (Ross JB et al., 1996) have been developed. These include phenothiazine type compounds such as chlorpromazine and butyrophenone derivatives, and benzamide type compounds such as sulpiride. The newer antipsychotic drugs, without substantial risk of extra pyramidal effects (John L Neumeyer et al., 2000) are referred to as atypical neuroleptics. The term atypical refers to the mechanism of antipsychotic action other than or in addition to postsynaptic D2 receptor blockade. Atypical agents are clozapine, Risperidone, Aripiprazole, Quetiapine, and Olanzapine (Umit B et. al, 2007).

Clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine) is classified as an 'atypical' antipsychotic drug (Ioulia Savina et al., 2007). It is a yellow powder with a molecular weight of 320.83 and melting point of 182°C. It is synthesized using very routine synthetic procedures. However, the current existing procedures of the synthesis involve the use of a few toxic chemicals and tedious workup processes. In this study, we aimed to develop a newer synthesis for Clozapine. The second objective of this project was to conduct impurity profiling on the newly developed synthetic process. Recently the pharmacopoeiae are specifying the nature of impurities in the monograph of drug products and the dosage manufacturers are also intended to have the impurity profile of the drug substance (Sharp TR et al., 2001, ICH., 2000). Therefore it was proposed to study the impurity profile of Clozapine (identifying the impurities by HPLC methods), synthesis and characterization of these impurities by spectral data and screening of the impurities (Alsante KM et al., 2001) for neuropharmacological activity along with the Clozapine.

Materials and Methods

All the chemicals used were of synthetic grade and acquired from Sigma-Aldrich distributor. Melting points were recorded on Polon melting point apparatus in open capillary tubes and were uncorrected. Reactions were monitored with the aid of TLC precoated silica gel G plates. The IR spectra were recorded on Perkin Elmer 882
FT-IR spectrophotometer by using 1% KBr technique and
H\textsuperscript{1}-NMR spectra are obtained in CDCl\textsubscript{3} on Brukar
Avance 300 MHz relative to TMS as internal standard
respectively. The Electron ionization mass spectrum was
recorded on Agilent 1100 series LC-MSD-TRAP-SL
system. Purity of the compounds was determined by the
Waters HPLC system using C-18 column. Pharmacological
evaluation was carried out on adult male albino mice (20-30 g). These were procured from Mahaveer Enterprises,
Hyderabad, India. The animals were fed with standard
pellet diet and water ad libitum. All the animals were
acclimatized a week before use. The locomotor activity
was determined by Digital Photoactometer (M.K.M.,
Chennai). Chemicals used were Clozapine (Clozaril\textsuperscript{®},
Novartis), Clozapine related compounds, and dimethyl
sulphoxide (DMSO) are of standard grades.

Scheme-I

Proposed route of synthesis

\begin{align*}
\text{TiCl}_4 & \quad \text{Toluene, anisole} \\
& \quad \text{Reflux for 3 hrs} \\
8\text{-chloro-11oxo-10,} \\
& \quad \text{11-dihydro-5H-dibenzo-1,} \\
& \quad \text{4-diazepine} \\
1\text{-Methylpiperazine} & \quad \text{Reflux for 4 hrs} \\
& \quad \text{CLOZAPINE (crude)} \\
& \quad \text{Recrystallization from acetone-IPE} \\
& \quad \text{CLOZAPINE}
\end{align*}
Scheme-II

Synthesis of Clozapine Dimer and Desmethyl Clozapine

8-chloro-11-oxo-10, 11-dihydro
5H-dibenzo-1, 4-diazepine

\[ \text{Clozapine dimer} \]

Scheme-III

Synthesis of Clozapine N-Oxide

Synthesis of Clozapine and its related compounds

Clozapine

Scheme-I was used for the synthesis of Clozapine. In the Scheme-I, Toluene, 200 ml, anisole 20 ml and 8-Chloro-11-oxo-10, 11-dihydro 5H-dibenzo-1, 4-diazepine (10 g) charged in round bottom flask at RT and stirred for 30 min. The reaction mass cooled to 10-15°C and 9.7 ml (16.4 g) TiCl₄ was added drop wise for 30 mins with constant stirring. The reaction mixture was heated to 110-112°C and heated under reflux for 3 hr. The reaction mass was then
cooled to 10°C. Methyl piperazine 20 g was added to the reaction mass during next 30 min, and further it was continuously stirred for 30 mins at 10°C. The reaction mixture was then heated under reflux for 4 hr. Progress of reaction was checked by thin layer chromatography (TLC) using mobile phase chloroform and methanol (9:1). After confirmation the reaction mass was distilled off to get the residue. The residue was partitioned between ethyl acetate and 2M aqueous hydrochloric acid (300 ml each) and filtered under vacuum. Both layers were separated and the aqeous layer was washed with ethyl acetate (2X150ml) and basified with sodium hydroxide solution to pH 14. The basified aq. layer extracted with ethyl acetate (3X150ml). Ethyl acetate layer was washed with DM water (2X100ml) and then with brine solution (100ml) and dried over enhydrous sodium sulphate. The ethyl acetate layer was completely distilled off under vacuum below 40°C to get clozapine. This method has advantages over the previous methods in terms of time, solvent consumption and safety.

**Impurities**
1) 8-Chloro-11-oxo-10, 11-dihydro-5H-dibenzo-1, 4-diazepine, 2) Clozapine Dimer, 3) N-desmethyl clozapine or Norclozapine, 4) Clozapine N-oxide (Capuano et al., 1999, 2002) are the impurities in this synthesis of Clozapine.

**Preparation of Clozapine Dimer and Desmethyl clozapine**
In Scheme-II, of Toluene 200ml, anisole 20ml and 8-Chloro-11-oxo-10, 11-dihydro-5H-dibenzo-1, 4-diazepine (10 g) charged in round bottom flask at RT and stirred for 30 min. The reaction mass cooled to 10-15°C and TiCl4 9.7 ml (16.4 g) added drop-wise over 30 min. with stirring. The reaction mixture was heated to 110-112°C and refluxed for 3 hr. The reaction mixture cooled to 10°C. piperazine (25 g) was added to the reaction mass over 30 min. for desmethyl clozapine, for clozapine dimer, (15 g) and continued the stirring for 30 min. at 10°C. The reaction mixture heated under reflux for 4 hr. Progress of the reaction checked by thin layer chromatography (TLC) using mobile phase chloroform and methanol (9:1). After confirmation by TLC, reaction mass completely distilled off to get residue. The residue partitioned between ethyl acetate and 2M aqueous hydrochloric acid (300 ml each) and filtered under vacuum. Both layers separated and the aqeous layer was washed with ethyl acetate (2X150ml) and basified with sodium hydroxide (NaOH) to pH 14. The basified aq. layer extracted with ethyl acetate (3X150ml). Ethyl acetate layer washed with DM water (2X100ml) and then with brine solution (100ml) and dried over enhydrous sodium sulphate. The ethyl acetate layer completely distilled off under vacuum below 40°C to get the clozapine.

**Synthesis of Clozapine N-oxide**
In Scheme-III, Clozapine (10 g) and MDC (200ml) were charged into round bottom flask at RT, with stirring. The reaction mixture was cooled to 0°C and meta-chloro perbenzoic acid (mCPBA 15g) in methylene dichloride (MDC) drop-wise over 1 hr with stirring. Progress of the reaction checked by thin layer chromatography (TLC) with mobile phase consisting of chloroform and methanol (9:1 ratio). Temperature was raised to 15°C and pH adjusted to 7 by saturated sodium bicarbonate solution and both layers were separated. MDC layer was washed with DM water (2X200ml) dried over enhydrous sodium sulphate. The MDC layer completely distilled off under vacuum below 40°C.

**Table 2. Spectral data Analysis for Clozapine and its impurities**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IR (cm⁻¹)</th>
<th>NMR (in CDCl3, δ)</th>
<th>Mass (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>2968(aliphatic C-H str of CH3), 3260(NH str), 1504(C=N str), 1255 (C-N ben), 778 (C=C str)</td>
<td>7.2-6.5 (m, aromatic 7H), 4.8 (s, H of NH), 3.4-2.5 (t, 8H of CH2), 2.3 (t, 3H of CH3)</td>
<td>327(M+), 329(M+2)</td>
</tr>
<tr>
<td>Imp-A</td>
<td>3059 (aliphatic C-H str of CH3), 3181 (NH str), 1220 (C-N ben), 749 (C-C str)</td>
<td>7.2-6.5 (m, aromatic 7H), 4.8 (s, H of NH)</td>
<td>245 (M+), 511 (dimer), 753 (trimer)</td>
</tr>
<tr>
<td>Desmethyl clozapine</td>
<td>3257 (NH str), 1602 (C=N str), 1296 (C-N ben), 759 (C=C str)</td>
<td>7.3-6.5 (m, aromatic 7H), 4.89 (s, 2H of NH), 3.5-3 (t, 8H of CH2)</td>
<td>313(M+), 315(M+2)</td>
</tr>
<tr>
<td>Clozapine dimer</td>
<td>2920 (aliphatic C-H str of CH3), 3277 (NH str), 1599 (C=N str), 1251 (C-N ben), 757 (C=C str)</td>
<td>7.3-6.6 (m, aromatic 7H), 4.89 (s, H of NH), 3.5 (t, 8H of CH2)</td>
<td>539(M+), 541(M+2), 542(M+3)</td>
</tr>
</tbody>
</table>
Pharmacological Evaluation

Evaluation of Neuropharmacological activity

Clozapine is an antipsychotic drug act by decreasing the levels of dopamine, by antagonizing the dopamine receptors in central nervous system. It binds to not only dopamine receptors but also to various receptors in brain and elicits different neurological effects. Clozapine and the impurities are screened for the locomotor activity (Jelveh Lameh et al., 2007).

Locomotor Activity

Adult male albino mice (20-30 g) were divided into 3 groups. Group I (control) animals were administered only vehicle (DMSO), 0.5ml, i.p. Group II (standard) animals were administrated (i. p.) drugs, Clozapine in a dose of 2.5 mg/kg of body weight (Prus AJ et al., 2005). Group III-IX animals were administered by the impurities, through i.p. in 2.5 and 5 mg/kg body weight. All the groups of animals were tested after 10 min. for activity in photoactometer for 10 min. The basal activity scores of all animals were taken. Clozapine (0.5mg/kg, i.p.) injected and after 10 min re-tested each mouse for activity scores for 10 min. Difference in the activity, before and after drug administration calculated in terms of percentage decrease in motor activity (Khan et al., 2004).

All the impurities have decreased the scores in the actophotometer compared to the 0th minute (before administration of the impurities) indicating that they exhibit the locomotor activity similar to that of clozapine at both the tested doses (2.5 mg& 5 mg/kg); desmethyl clozapine being more closer to clozapine. The scores and the percentage inhibition of the locomotor activity are presented in Table 3 and 4 respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>6.0±3.13</td>
</tr>
<tr>
<td>II</td>
<td>Clozapine</td>
<td>2.5</td>
<td>48.5±3.6*</td>
</tr>
<tr>
<td>III</td>
<td>Imp-A</td>
<td>2.5</td>
<td>30.2±3.13*</td>
</tr>
<tr>
<td>IV</td>
<td>Imp-A</td>
<td>5</td>
<td>48.8±2.57*</td>
</tr>
<tr>
<td>V</td>
<td>Desmethyl Clozapine</td>
<td>2.5</td>
<td>46.1±6.3*</td>
</tr>
<tr>
<td>VI</td>
<td>Desmethyl Clozapine</td>
<td>5</td>
<td>49.4±6.6*</td>
</tr>
<tr>
<td>VII</td>
<td>Clozapine-N-oxide</td>
<td>5</td>
<td>8.3±4.14</td>
</tr>
<tr>
<td>VIII</td>
<td>Clozapine dimer</td>
<td>2.5</td>
<td>47.4±5.12*</td>
</tr>
<tr>
<td>IX</td>
<td>Clozapine dimer</td>
<td>5</td>
<td>49.2±1.75*</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6); * p<0.001 Vs Control
Discussion

Clozapine is a dibenzodiazepine derivative having neurotransmission blocking activity for dopaminergic receptors and used in the treatment of schizophrenia. The prior methods of synthesis of Clozapine involve the use of toxic chemicals and tedious workup procedures. In one of the reported synthetic process (US Patent – 3962248), ether is used for extraction and ammonia for basification. In another process (US Patent – 3539573) phosphoroxycloride and benzene are used. This present synthetic process, it avoid the use of above toxic chemicals and makes use of less toxic and safe chemicals like TiCl₄ and ethyl acetate. The impurity profiling for this synthetic process by HPLC has revealed four impurities viz; starting material (8-chloro-11-oxo-10, 11-dihydro-5H-dibenzo-1, 4- diazepine). Clozapine dimmer, Desmethyl Clozapine and Clozapine N oxide. All these impurities are structurally very close to clozapine. Therefore, these impurities were also synthesized and evaluated for locomotor activity along with clozapine, with a view to find whether they also possess similar activity. The locomotor activity screening has shown that all the impurities possess similar kind of activity. However, the magnitude of the activity is less than clozapine except the desmethyl clozapine which is almost equal to clozapine. Therefore, it can be concluded that the presence of these impurities in the clozapine bulk active chemical do not alter the pharmacological profile of the clozapine drug to any greater extent.

Acknowledgments

The authors are thankful to G. Pulla Reddy College of Pharmacy, Hyderabad, AICTE, New Delhi, and Matrix Laboratories, Hyderabad.

References


Umut B. Semiz, Mesut Cetin, Cengiz Basoglu, Servet Algul and Alpay Ates, Clinical predictors of therapeutic response to clozapine in a sample of Turkish patients with treatment-resistant schizophrenia. Progress in Neuro-Psypharmacology and Biological Psychiatry. 31(6):1330-1336(2007).


Prus AJ, Philhbin SD, Pehrson, AL, Porter JH. Generalization to atypical antipsychotic drugs depends on training dose in rats trained to discriminate 1.25 mg/kg clozapine versus 5.0 mg/kg clozapine versus vehicle in a three-choice drug discrimination task. Behavioural Pharmacology. 16(7): 511-528(2005).


