Synthesis, structural elucidation and reaction optimization studies of a novel prodrug of Atovaquone

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Abstract. In the present work, a novel prodrug has been synthesized to enhance the therapeutic efficacy of a renowned antimalarial drug, Atovaquone. Prodrug of the present work overcomes the challenges associated with the poor solubility of the parent drug, Atovaquone and thus contributes to increased drug bioavailability. The present study summarizes the synthesis, characterization and reaction condition optimizations in a systematic pathway. The present disclosure provides a consistent and repeatable reaction conditions for the synthesis of Atovaquone prodrug in high yields and good purity. Compact reaction optimization studies enable the synthetic process to be suitable for large scale manufacturing, a significant initiative towards commercialization of the prodrug.

Keywords. Atovaquone; Prodrug; Solubility; Synthesis; Structural elucidation; Reaction optimization; Metabolite.

1. Introduction

The well known antimalarial drug, Atovaquone 1 exhibits very low bioavailability, when administered orally due to its poor solubility. To achieve better therapeutic efficacy, 1 is usually combined with Proguanil for the treatment.1–3 Extreme lipophilic nature of 1 makes it to display very low water solubility.4,5 Drug solubility can be enhanced by various strategies like particle size lowering, salt preparation, prodrug pathway, use of surfactants, cyclodextrin complexation, lipid influenced delivery systems, solid dispersion etc. An easier approach to enhance the drug solubility is by converting the parent drug to a prodrug having better solubility. Therapeutically administered prodrug gets metabolized and releases the parent drug. This innovative drug administration strategy has been employed to improve the ADME of low solubility drugs.6 The use of better soluble prodrug avoids the high dose administration of the parent drug for the treatment. The prodrug methodology can be effectively used to address the issues related to solubility, permeability, stability, pre-systemic metabolism, target limitations etc.7 Prior art disclosures towards the synthesis and applicability of Atovaquone prodrugs are tabulated systematically to introduce 3 as a novel prodrug with better solubility and superior clinical applicability.

A study was conducted on the synthesis, characterization and drug efficacy estimation of a novel
prodrug 1.1. It had exhibited better bioavailability than 1 and had shown enhanced efficacy towards Pneumocystis carinii pneumonia. A study reported few analogues of 1 (hydrogen of –OH group was replaced by the substituted ester and ether groups) such as 1.2 and 1.3 series were disclosed with details on the synthesis, characterization and antimalarial activity. All the molecules have displayed better biological activity than Chloroquine. A study reported the prodrugs 1.4 to 1.7, which were designed by an innovative computational approach. Based on this venture, drug release from its prodrug could be systematically predicted based on the nature of the linker. Few of the new prodrugs 1.8 to 1.12 which are more hydrophilic than 1 were reported with their design was based on the computational strategy. This non-enzyme-mediated disclosure had a controlled drug release capability in a sustainable manner. Based on the information gathered from Bruice’s model, 1.11 was synthesized by the reaction of 1 with Succinic anhydride in the presence of a strong base like Sodium Hydride (NaH), isolated and characterized. A dissolution study of the prodrug was conducted at different buffer conditions to gather its solubility and hydrolysis data. These initiatives to introduce 1.11 gave more promising results than 1 in terms of drug efficacy, physicochemical properties and the rate of drug release. The computational methodology of prodrug design based on the enzyme models, will assist in the prediction of cleavage associated with drug release. The prodrug cleavage happens by chemical reactions like hydrolysis, oxidation etc to release the active parent moiety. An intense study in these regards will facilitate a better understanding of the enzyme catalysis concepts and the knowledge will roll on to develop efficient prodrugs. A progressive review report on the prodrugs of Atovaquone and Buparvaquone, emphasizes the prior art disclosures on the synthesis and therapeutic advantages of 1.1 to 1.12 and 3.
2. Materials and methods

Present work involves the conversion of a low water-soluble but active pharmaceutical ingredient 1 to a better soluble novel prodrug 3. The parent drug 1 was prepared by an innovative synthetic procedure using 2, 3-dichloro-1,4-naphthoquinone (Dichlone) as the key starting material.16 The reagent, 5-methyl-4-chloromethyldioxalone 2 was procured from Loba-chem, potassium carbonate from Rankem and the commercial solvents were procured locally. These procured reagents and solvents were used in the experiments without further purification.

The melting point (m.p) of the compound was recorded by the open capillary method and is uncorrected. 1HNMR spectra were recorded (in DMSO-d6/CDCl3) on a 400MHz NMR spectrometer using Tetramethylsilane (TMS) as an internal reference standard. Coupling constants J are in Hz and multiplicities are represented as a singlet (s), doublet (d), triplet (t), broad singlet (bs) and multiplet (m). Mass spectra were recorded on Agilent mass spectrometer operating at 70ev. Progress of the reactions and the purity of the product was checked by thin-layer chromatography (TLC) using pre-coated silica TLC plates (Merck 60F254) and HPLC.

2.1 Synthesis and characterization of 3

Parent drug 1 (30.00 g, 0.0818 mol), potassium carbonate (28.26 g, 0.2045 mol) and 600 mL of acetonitrile were taken in the reactor, which was fitted with a guard tube. Added 2 (30.38 g, 0.2045 mol) dropwise to the reaction mixture under stirring at room temperature (RT). The reaction mass was heated to 60–65 °C and maintained under stirring at the same temperature for around 16–18 h. Reaction progress was monitored by TLC. After the reaction completion, the solvent was removed completely by vacuum distillation to isolate the crude 3 as a sticky residue. To the obtained residue, added 450 mL aqueous solvent (300 mL of water and 150 mL of ethyl acetate) and stirred at RT for 30 min. Filtered the mass under suction and dried at 65–70 °C. The elemental analysis (C, H, N) data, Theoretical: C: 67.65%, H: 4.80%, Obtained: C: 67.03%, H: 4.91%. Mass data (Cl35), m/z: 478.0. Purity by HPLC: 99.0%.

2.2 Experiments for the selection of reagents and solvents

Synthesis of 3 was carried out by using different combinations of reagents and solvents to achieve better yield and purity (Table 1). Various reagent (base) and solvent combinations were used distinctively for the experiments, such as sodium carbonate and tetrahydrofuran, potassium carbonate and N, N-dimethylformamide, potassium carbonate and dimethylsulphoxide, potassium carbonate and N, N-dimethylacetamide and pyridine and chloroform. All the experiments were done by taking 1 (5.00 g), base (2.0 equiv) and solvent (50 mL). However, none of the above combinations (Exp. No. 1–5) resulted in satisfactory results like the efficient combination of potassium carbonate and acetonitrile (Exp. No. 6) with regard to yield and purity of 3.

2.3 Experiments to optimize the quantity of reagents and solvents

Synthesis of 3 was carried out by varying the input quantity of 2 in the presence of potassium carbonate and solvent acetonitrile. The quality (by HPLC) and quantity (yield) of isolated 3 was analyzed (Table 2). It was evident from the experimental results that, good yield and better quality (Exp. No. 4 and 6) was obtained upon using 2 (2.5 equiv) and isolation of 3 using 15v of (water and ethyl acetate) mixture in the ratio of (2:1). Lower input of 2 for the reaction resulted in significantly lower yields. The use of an aqueous solvent (water and ethyl acetate), featured in the isolation stage has a vital impact on the effective removal of inorganic salts and the polar impurities rather than the use of mixture (water and acetonitrile). The studies towards reaction optimization were carried out by conducting experiments with 10.00 g input of 1 (Expt. No. 1–5) and the reaction was scaled up to 30.00 g input of 1 to confirm the reaction reproducibility towards consistent yield and purity (Expt. No. 6).
Table 1. Experimental details to synthesize 3 using various reagents and solvents

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Reagents and reaction conditions</th>
<th>Reaction progress (by TLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sodium carbonate and tetrahydrofuran, refluxed for 10 h.</td>
<td>No product formation.</td>
</tr>
<tr>
<td>2.</td>
<td>Potassium carbonate and N, N-dimethylformamide, 80–85 °C for 15 h.</td>
<td>No product formation.</td>
</tr>
<tr>
<td>3.</td>
<td>Potassium carbonate and dimethylsulphoxide, 80–85 °C for 10 h.</td>
<td>Less product formation (around 10%).</td>
</tr>
<tr>
<td>6.</td>
<td>Potassium carbonate and acetonitrile, 60–65 °C for 16–18 h.</td>
<td>Product formed (around 85%) with less impurities.</td>
</tr>
</tbody>
</table>

3. Results and Discussion

3.1 Chemistry

The phenolate anion of 1 formed in presence of potassium carbonate displaces the reactive chlorine in 2 to form 3 (Scheme 1). Since compound 1 offers substantial steric hindrance, higher input of reagent 2 (2.5 equiv) was required along with a prolonged reaction time of around 16–18 h at 60–65 °C. Due to the longer reaction time, there is a progressive rise in the formation of polar impurities during condensation. To remove the polar impurities and the inorganics formed during the synthesis of 3, a slurry wash step has been introduced after the isolation of crude 3. This step involves the use of 15v of (water and ethyl acetate) mixture in the ratio of (2:1) at RT. Product 3 was isolated by the filtration followed by drying at 65–70 °C under reduced pressure for 4 h. The in vitro hydrolysis of 3 will release the clinically active parent drug 1 and the corresponding metabolite 4. The resultant byproduct 4 (Medoxomil alcohol) is a proven non-toxic metabolite released even during the in vitro hydrolysis of Olmesartan Medoxomil (antihypertensive drug). The cleavage rate of ether linkage is relatively slower compared to that of ester linkage prodrugs.

The parent drug 1 has poor water solubility, as per the prior art disclosure, it has a reported solubility of 0.74 µg/mL. The novel prodrug 3 of present invention exhibited an improved water solubility of 3.75µg/mL. This enhanced water solubility of 3 will have a significant contribution towards the bioavailability and clinical efficacy of 1.

3.2 Structural elucidation

The structure of 3 was established based on the 1HNMR, mass spectrum, IR and elemental analysis (C, H, N) data. The mass spectrum of 3 (Figure S1, Supplementary Information) showed a molecular ion peak with m/z values of 478 (Cl35) and 480 (Cl37) with molecular weight of 478.92. The IR spectrum of 3 (Figure S2, Supplementary Information) showed an absorption band at 2925.87 cm⁻¹ was assigned for the C–H stretching (–O–CH2). The absorption band at 1826.47 cm⁻¹ was for C=O of dioxolane group. The absorption band at 1669.47 cm⁻¹ is for the C=O group of naphthoquinone ring. The 400 MHz 1HNMR spectrum of 3 (Figure S3a and Figure S3b, Supplementary Information) showed two doublets (J = 7.56Hz) in the range δ 8.02–8.10 ppm was assigned for 2 protons attached to C5 and C8. The multiplet in the range δ 7.69–7.76 ppm was assigned for 2 protons attached to C6 and C7. Aromatic protons which resonated as a doublet (J = 7.68Hz) in the range δ 7.25–7.27 ppm integrated for 2 protons which are attached to C24 and C26. Aromatic protons which resonated as a doublet (J = 8.4Hz) in the range δ 7.17–7.19 ppm integrated for 2 protons which are attached to C23 and C27. The singlet at δ 5.22 ppm was assigned for 2 protons attached to C11. The multiplet in the range δ 3.16–3.24 ppm was assigned for one proton attached to C19. The chemical shifts and shapes of the tertiary protons on the cyclohexyl ring at about δ 3.2 and 2.6 ppm were almost identical to those of 1 showing that the geometry had not been disturbed during the formation of 3. The singlet at δ 2.19 ppm is for the 3 protons attached to C15. The remaining 8 protons attached to C17, C18, C20 and C21, respectively, were resonated as multiplets in the range δ 1.50–2.17 ppm. The elemental analysis (C, H, N) report of 3 (Figure S4, Supplementary Information) showed 67.03% of Carbon and 4.91% of Hydrogen content (Theoretical: C: 67.65%, H: 4.80%).
Table 2. Details of reaction optimization studies conducted to isolate 3 in high yields and better purity.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Experimental conditions/pathway</th>
<th>Yield</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 (10 g, 0.0273 mol), potassium carbonate (5.66 g, 0.0409 mol) and 200 mL of acetonitrile. Added 2 (4.05 g, 0.0273 mol) and heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 150 mL of aqueous solvent (100 mL of water and 50 mL of acetonitrile).</td>
<td>45.11%</td>
<td>91%</td>
</tr>
<tr>
<td>2</td>
<td>1 (10 g, 0.0273 mol), potassium carbonate (5.66 g, 0.0409 mol) and 200 mL of acetonitrile. Added 2 (6.09 g, 0.0409 mol) and heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 150 mL of aqueous solvent (100 mL of water and 50 mL of acetonitrile).</td>
<td>62.80%</td>
<td>93%</td>
</tr>
<tr>
<td>3</td>
<td>1 (10 g, 0.0273 mol), potassium carbonate (9.43 g, 0.0683 mol) and 200 mL of acetonitrile. Added 2 (10.14 g, 0.0683 mol) and heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 150 mL of aqueous solvent (100 mL of water and 50 mL of acetonitrile).</td>
<td>72.12%</td>
<td>99%</td>
</tr>
<tr>
<td>4</td>
<td>1 (10 g, 0.0273 mol), potassium carbonate (9.43 g, 0.0683 mol) and 200 mL of acetonitrile. Added 2 (10.14 g, 0.0683 mol) drop wise at 25–30 °C. Heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 150 mL of aqueous solvent (100 mL of water and 50 mL of ethyl acetate).</td>
<td>86.27%</td>
<td>98.9%</td>
</tr>
<tr>
<td>5</td>
<td>1 (10 g, 0.0273 mol), potassium carbonate (9.43 g, 0.0683 mol) and 200 mL of acetonitrile. Added 2 (10.14 g, 0.0683 mol) drop wise at 25–30 °C. Heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 100 mL of aqueous solvent (50 mL of water and 50 mL of ethyl acetate).</td>
<td>61.50%</td>
<td>98.5%</td>
</tr>
<tr>
<td>6</td>
<td>1 (30 g, 0.0818 mol), potassium carbonate (28.26 g, 0.2045 mol) and 200 mL of acetonitrile. Added 2 (30.38 g, 0.2045 mol) drop wise at 25–30 °C. Heated the mass to 60–65 °C for 16–18 h. Isolation was done by using 450 mL of aqueous solvent (300 mL of water and 150 mL of ethyl acetate).</td>
<td>90.00%</td>
<td>98.7%</td>
</tr>
</tbody>
</table>

Scheme 1. Synthesis of 3 and its hydrolysis to release 4

4. Conclusions

The present study discloses a novel Atovaquone pro-drug 3, with a focus to enhance the bioavailability and therapeutic efficacy of a renowned antimalarial drug, Atovaquone 1. The challenges associated with poor solubility and variable bioavailability of 1 had led us to the synthesis of 3, which has better water solubility. In this paper, we summarize the synthesis, characterization and reaction optimization studies of 3. The synthesis involves the use of commonly available and cost viable key raw material 2. The synthetic pathway does not involve the use of any kind of toxic or hazardous chemicals, even the released moiety 4 from 3 was proven to be a non-toxic metabolite. The systematic reaction optimization studies would enable the commercialization of 3 with maximum yield and high purity.
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Supplementary Information

The spectral details which are supportive to the systematic characterization aspects of novel prodrug are \( \text{Figure S1 to S4, from pages no. 3 to 6} \) available at \( \text{www.ias.ac.in/chemsci} \).

References

3. Shaji J and Bhatia V 2013 Dissolution enhancement of atovaquone through cyclodextrin complexation and phospholipid solid dispersion \( \text{J. Int. Pharm. Pharm. Sci. 5} \) 642
4. Nicolaides E, Galia E, Ethymiopoulos C, Dressman J B and Reppas C 1999 Forecasting the in vivo performance of four low solubility drugs from their in vitro dissolution data \( \text{Pharm Res. 16} \) 1876
7. Hong W, Huijeong J and Li X 2015 Drug Delivery Approaches in Addressing Clinical Pharmacology-Related Issues: Opportunities and Challenges \( \text{AAPS J. 17} \) 1327
15. Sanjay S S, Shridhara K and Shashiprabha 2021 A review on atovaquone and buparvaquone prodrugs \( \text{World J. Pharm. Res. 10} \) 2108
18. Petra L, Kurt P and Wilhelm K 2001 The pharmacokinetic and metabolic profile of olmesartan medoxomil limits the risk of clinically relevant drug interaction \( \text{J. Hypertens. 19} \) 21