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Review

Chemical Structure and Synthesis of Baloxavir Marboxil, A Novel Endonuclease Inhibitor For The Treatment Influenza : An Overview

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ABSTRACT: One drug target that was identifed as a promising candidate for infuenza antiviral drugs is the infuenza virus polymerase complex. The three-component polymerase complex, which is highly conserved and is essential for infuenza virus replication, has received considerable attention as a potential target for infuenza antiviral drugs. Baloxavir marboxil (BXM, Xofuza®; hereafter referred to as baloxavir), the prodrug of baloxavir acid, is a frst-in-class, small molecule inhibitor of the cap-dependent endonuclease reaction that is conducted by the polymerase acidic (PA) protein subunit of the infuenza virus polymerase complex. In this review, we summarized the chemical structure, synthesis, mechanism of action, pharmacological activity and synthesis of Baloxavir marboxil, a novel FDA-approved endonuclease inhibitor for the treatment of influenza.

Keywords *:* Antiviral, baloxavir marboxil, endonuclease inhibitor, influenza, synthesis.

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1. INTRODUCTION

The influenza virus (IV) causes an infectious disease associated with 290,000 to 650,000 deaths and 3 to 5 million cases of severe illness worldwide each year [1]. Additionally, pandemics caused by emerging reorder viruses can have a devastating impact globally. Therefore, continuous efforts are needed to improve the vaccines and antiviral drugs used in the treatment [2]. Two classes of antivirals are currently available for clinical use, neuraminidase inhibitors (NAIs: oseltamivir, zanamivir, peramivir) and M2 ion channel inhibitors (amantadine, rimantadine) [3]. However, influenza viruses are now largely resistant to M2 inhibitors. Furthermore, the antiviral potency of NAIs is relatively moderate, and another concern for this class of drugs is the emergence of resistance, as occurred during the 2008 to 2009 season when oseltamivir-resistant H1N1 was prevalent [4-7]. Therefore, more effective antiviral agents with a new mechanism of action are required for the treatment and prevention of influenza virus infections [7].

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Baloxavir is the first antiviral drug in the cap-dependent endonuclease inhibitors class. Currently, the only FDA-approved indication is the treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for more than 48 hours. Treatment consists of an oral dose within the first 48 hours of illness onset [8]. Shionogi discovered and developed baloxavir marboxil and received approval in Japan in February 2018 [8]. Shionogi transferred its rights to Roche / Genentech, Inc. on May 17, 2018, with approval in the United States in October 2018 [8]. The general properties of baloxavir marboxil are given in Table 1 [9].

Table 1. General properties of baloxavir marboxil compound

Generic Name	Baloxavir Marboxil
Trade Name	Xofluza
CAS Number	1985606-14-1
Formula	$C_{27}H_{23}F_2N_3O_7S$
Molecular Weight	571.55 $g \cdot mol^{-1}$
Mechanism of Action	Endonuclease inhibitors
Class	Antivirals; Dibenzothiepins; Esters; Pyridines; Triazine
Application Path	Oral
Recommended Indication	Treatment of acute uncomplicated influenza in patients 12 years of age and older who have been symptomatic for no more than 48 hours

1.1. General Chemical Structure of the Compound

Baloxavir marboxil is a polycyclic carbamoylpyridone derivative compound [10]. The structure of the polycyclic carbamoylpyridone derivatives of the present invention is as in Figure 1. The polycyclic carbamoylpyridone derivative of the present invention functions as an inhibitor of influenza 5' cap-like structure (CAP)-dependent endonuclease activity and can be used to treat a cold caused by influenza virus [10, 11].

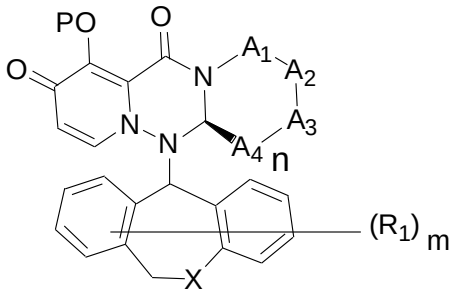
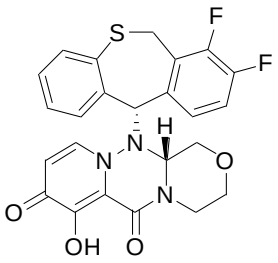


Figure 1. Structure of Polycyclic Carbamoylpyridone Derivatives

Baloxavir acid (BXA,1) is the active metabolite of the prodrug baloxavir marboxil (BXM, 2) which is marketed as Xofluza® (Figure 2). The IUPAC name of BXM is ({(12aR)-12-[(11S)-7,8-difluoro-6,11-dihydrodibenzo[b,e]thiepin-11-yl]-6,8-dioxo-3,4,6,8,12,12a-hexahydro-1H-[1,4]oxazino[3,4-c]pyrido[2,1-f][1,2,4]triazin-7-yl}oxy)methyl methyl carbonate [12]. Baloxavir marboxil is a prodrug that hydrolyzes to produce the active drug, baloxavir acid (Figure 2). At the 5 stages of a viral life cycle in the host cell (viral entry, uncoated, viral replication, assembly and budding, and finally viral release), baloxavir targets the third stage of viral replication [13].



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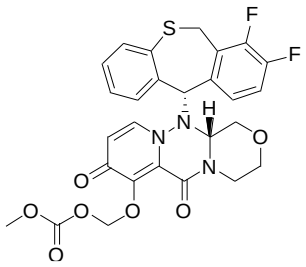
Baloxavir Acid (BXA)



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Baloxavir Marboxil (BXM)

Figure 2. Chemical structure of baloxavir acid (BXA, active form) and baloxavir marboxil (prodrug form).

1.2. Mechanism of Action

Influenza virus is a member of the RNA-virus group and humans are infected by three viral strains: IVA, IVB, and IVC. IV infects cells by binding sialic acid residues, which promotes its endocytosis via an enzymatic cascade. This virus, after its penetration and uncoating, RNA is replicated through a relatively complicated process [14]. Eight segments of viral RNA encode for 11 to 12 proteins only, depending on the strains [15]. RNA dependent RNA polymerase and other viral nucleoproteins are a molecular complex called the viral ribonucleoprotein complex [16]. This complex works on both replication of viral RNA to produce new virions and transcription to synthesize proteins. RNA dependent RNA polymerase is formed of different subunits: polymerase acidic protein (PA), polymerase basic protein 1 (PB1), and polymerase basic protein 2 (PB2) [17]. Baloxavir acid inhibits the endonuclease activity of polymerase acidic (PA) protein, one of the subunits of viral RNA polymerase. This endonuclease is a virus-specific enzyme required for viral gene transcription [12]. Transcription of viral mRNA begins via a "cap snatching" mechanism, binds to a capped RNA formed by the host's RNA polymerase II, and the endonuclease of the PA protein cleaves from the 5'-cap of the RNA to produce a primer [18]. This capped primer is then used by viral RNA polymerase for viral mRNA synthesis. The enzyme that produces this essential primer is called capsular endonuclease, and through inhibition of this enzyme, baloxavir can inhibit influenza viral replication for both influenza A and B viruses [19]. The antiviral mechanism of BXM is given in Figure 3 [18].

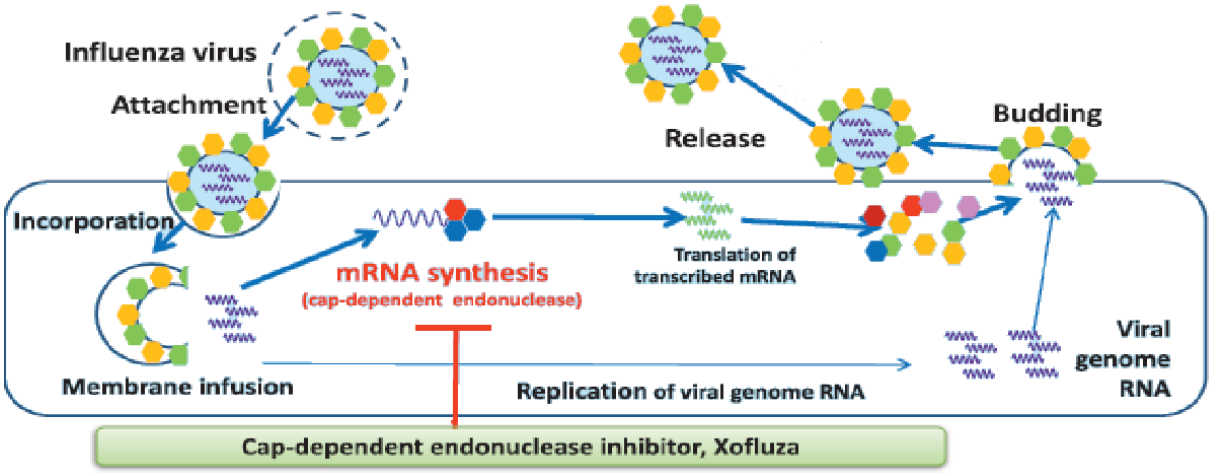

Figure 3. Antiviral mechanism of BXM

1.3. Pharmacokinetic Properties of Baloxavir Marboxil

Following oral administration of baloxavir marboxil, it is converted to the active metabolite, baloxavir, by esterases in the GI lumen, liver, and blood [20]. The mean time to reach plasma concentrations of baloxavir after oral administration of baloxavir marboxil is 4 hours [21]. Baloxavir and its metabolites are distributed in milk in rats, it is not known whether they are distributed in breast milk. The plasma protein binding of BXM is approximately 93-94% [22]. Following oral administration, baloxavir marboxil is rapidly hydrolyzed by esterases to its active metabolite baloxavir [20].

Baloxavir is metabolized via UGT1A3 and CYP3A4 with major contribution from UGT1A3. No clinically relevant effects were observed when baloxavir was co-administered with strong CYP3A and UGT inhibitors such as itraconazole and probenecid [23]. In addition, Baloxavir can chelate with polyvalent cations and therefore co-administration with drugs containing calcium, aluminum, magnesium or iron should be avoided [13, 23].

1.4. Synthesis of Baloxavir Marboxil

Baloxavir consists of two main parts, each with a chiral center. These two parts are shown in Figure 4. Each of these two parts (1,2) must be synthesized to obtain the resultant compound. The coupling and deprotection steps must then be performed [8, 24].

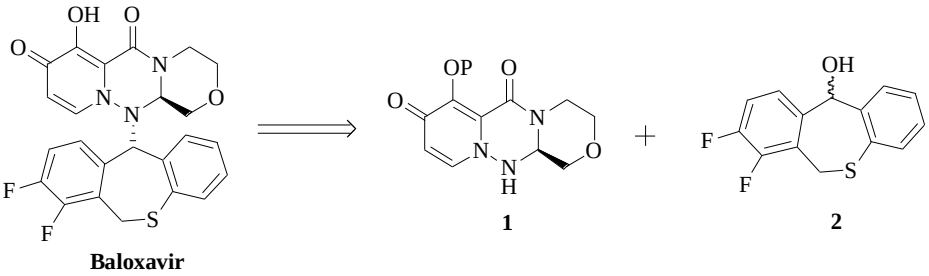

Figure 4. Retrosynthetic Approach to Baloxavir

a) Synthesis of compound 1:

Synthesis of compound 1 started from lactam 3. Protection of 3 with allyl chloroformate yielded compound 4 in 62% yield. The synthesis proceeded with a three-step reaction of morpholino compound 6 (Figure 5). Reduction of DIBAL-H (93% yield) and addition with MeOH under acidic conditions in 87% yield resulted in racemic methoxy compound 6 (Figure 5) [24].

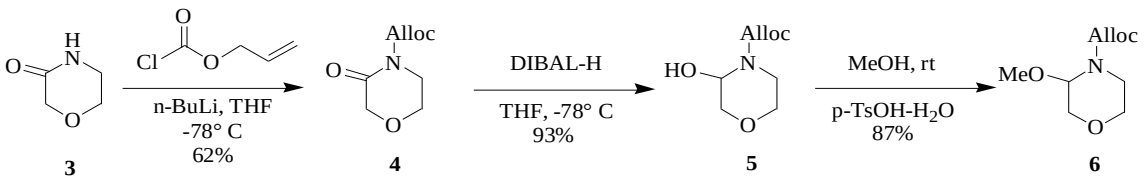

Figure 5. Preparation of Morpholino Compound 6

To prepare the hydrazine fragment (10) (1-amino-3-benzyloxy-4-oxo-1,4-dihydro-pyridine-2-carboxylic acid ethyl ester), the carboxylic acid-bearing compound 7 was esterified with EtI in quantitative yield and then It was reacted with Boc-hydrazine to produce pyridone 9 in 73% yield, followed by quantitative deprotection with anhydrous HCl (Figure 6) [24].

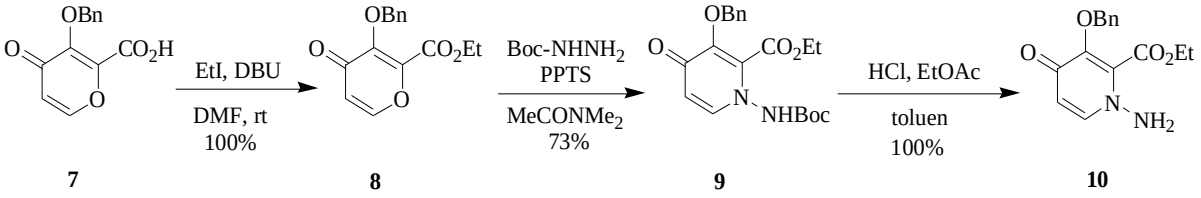

Figure 6. Preparation of Hydrazine Fragment 10

The combination of fragments 6 and 10 was mixed with $SnCl_4$ in MeCN at −25 °C to form the nonsubstituted hydrazine (11) (Figure 7). The Alloc protecting group was removed using Pd catalysis by cyclization to obtain compound 1-rac in 100% yield (Figure 7). Separation of the 1-rac enantiomers was accomplished by forming hydrazide diastereomers, selective crystallization of the desired (R, R)-diastereomer, and then hydrolysis to produce 1-R. Specifically, 1-rac was precipitated with diastereomers (R)-tetrahydrofuran-2-carboxylic acid (12) precipitated from the reaction mixture to form 13A and 13B. Removal of the tetrahydrofuroyl group was accomplished by addition of DBU in EtOH for 0.5 hours at room temperature. Then, by adding diisopropyl ether to the reaction mixture, 1-R was obtained in 90% yield (Figure 7) [24].

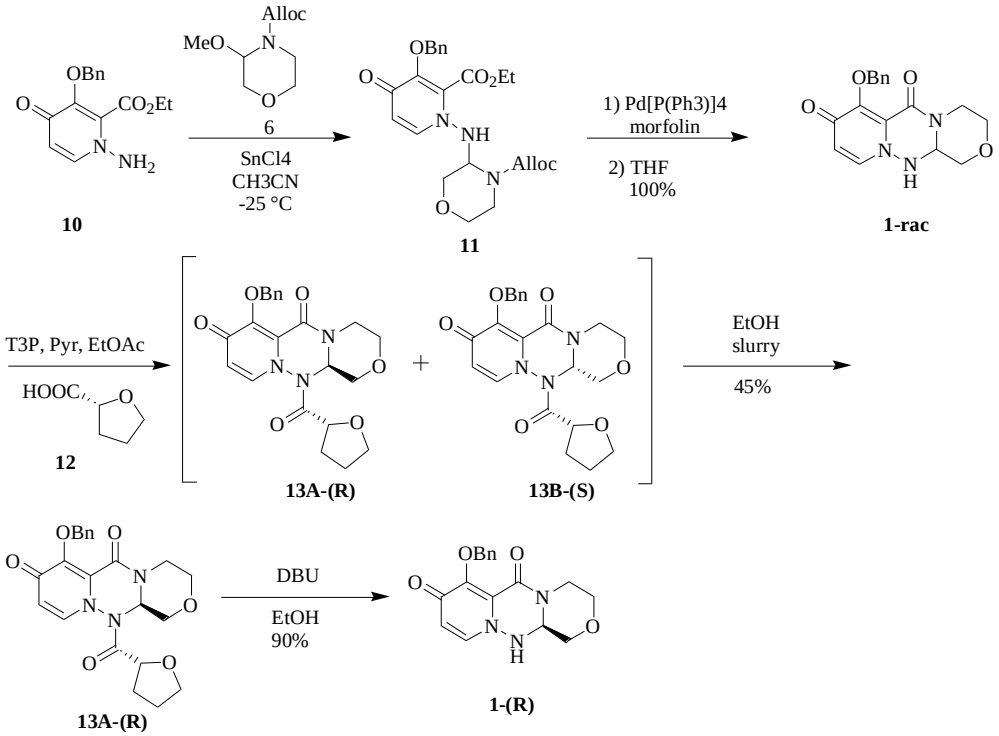

Figure 7. Preparation of compound 1

b) Synthesis of tricyclic sulfur (2) compound

The synthesis of the tricyclic sulfur compound (2) was prepared in five steps and in 71% yield (Figure 8). 3,4-Difluorobenzoic acid (14) was metallized at the 2-position between the carboxylic acid and the 3-F group using Lithium diisopropylamide (LDA) in tetrahydrofuran and then treated with DMF to form compound 15. After replacing the solvent with toluene, reaction with D-camphorsulfonic acid-mediated thiophenol gave thioacetal compound 16 as solution in toluene. Compound 16 was then reduced with 1,1,3,3-tetramethylsiloxane and $AlCl_3$ to obtain compound 17. Treatment of compound 17 with pure phenylpropanolamine (PPA) at 120 °C yielded compound 18, which is tricyclic sulfide in 91% yield. Reduction of the ketone was carried out with $NaBH_4$ to give racemic sulfide 2 in 97% yield [8, 24].

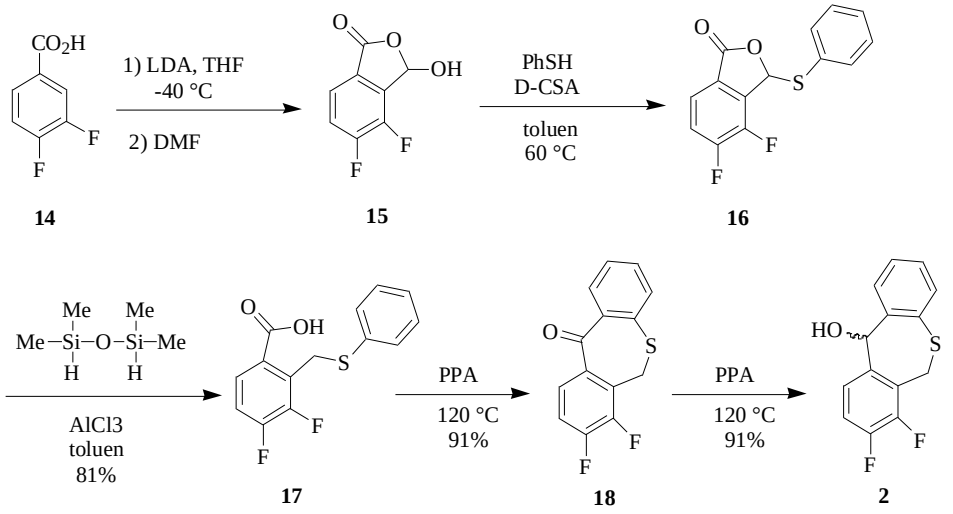

Figure 8. Preparation of Tricyclic Sulfite (2) compound

c) Final step for baloxavir marboxil synthesis:

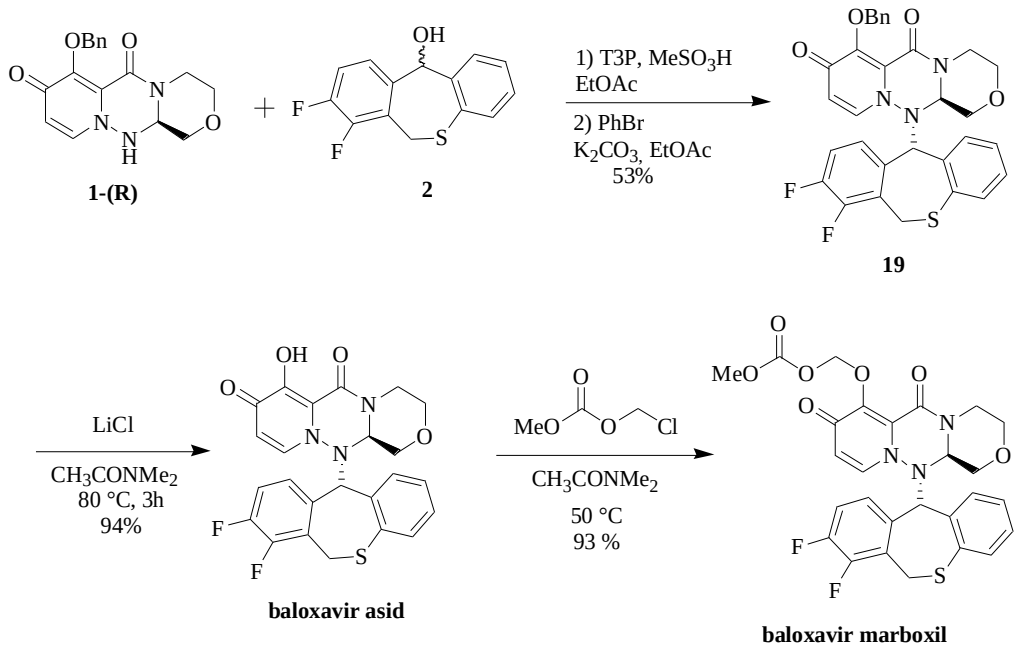

Figure 9. Final step for baloxavir marboxil synthesis

The coupling of the 1-R and 2 fragments was carried out under dehydration conditions of 1-propanephosphonic anhydride (T_3P) and methanesulfonic acid at 70 °C to obtain protected baloxavir 19. Compound 19 was then reacted with 0.6 equivalents of PhBr and K_2CO_3. Debenzylation was then carried out using LiCl in CH_3CONMe_2 to give baloxavir acid in 94% yield. In the final step for the preparation of the prodrug, baloxavir acid was reacted with chloromethyl methyl carbonate in dimethylacetamide in 93% yield to form baloxavir marboxyl. The reaction mechanism for the final step for the synthesis of BXM is given in Figure 9 [8, 24].

CONCLUSION

The approval of baloxavir marboxil as an influenza antiviral drug is a big step forward as it represents a new age class of drugs for the treatment of influenza, such as endonuclease inhibitors. The discovery, synthesis and proof of influenza inhibitory effect of BXM, a polycyclic carbamoylpyridone derivative, is important for the discovery of new endonuclease inhibitor antiviral agents with this special structure.

Conflict of Interest

Author has no personal financial or non-financial interests.

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