Pleuromutilin and its Derivatives-The Lead Compounds for Novel Antibiotics

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Abstract: Due to the rapid onset of resistance to most antibacterial drugs, research efforts are focusing on new classes of antibacterials with different mechanisms of action from clinically used antibacterials. Pleuromutilin derivatives have received more and more scientific attention for their unique mechanism of action. Two pleuromutilin derivatives, tiamulin and valnemulin have been successfully developed as antibiotics for veterinary use. Retapamulin, another pleuromutilin derivative has been approved for use in humans in April 2007 by Food and Drug Administration (FDA). It has been shown that there is rarely cross-resistance between pleuromutilin derivatives and other antimicrobial agents, and the development of resistance bacterial is still low. This review will demonstrate mechanism of action of pleuromutilin derivatives and reveal the structure-activity relationship (SAR) of pleuromutilin derivatives. Additionally, the pleuromutilin antibacterial derivative agents in the market, such as tiamulin, valnemulin and retapamulin, will be discussed. It is proposed that new antibacterial agents might be developed from pleuromutilin derivatives in the future.

Keywords: Pleuromutilin, antibiotic, structure-activity relationships, *in vivo*.

INTRODUCTION

The invention of sulphonamide antibiotics in 1930s and penicillin in 1940s significantly decreased the fatality rates associated with bacterial infections [1]. The invention of sulphonamide and penicillin led to search for other new antibacterial drugs which resulted in the discovery of series antibacterial drugs as known today, however, only three novel classes of antibacterial drugs had entered the market since 1970 [2]. With the increasing use of antibacterial drugs for infectious diseases, the emergence and spread of resistance to existing antibiotics became a major concern in medical community. Therefore, research efforts are focusing on new classes of antibacterial drugs with different mechanisms of action from clinically used ones.

The antibiotic pleuromutilin (**1**, Fig. **1**), with a fused 5-6-8 tricyclic diterpenoid structure, was first isolated in 1951 from two basidiomycete species [3]. This antibiotic was characterized as a crystalline antibiotic with modest antibacterial activity against Gram-positive pathogens and mycoplasma *in vitro* but weak activity in *in vivo* [4]. Pleuromutilin selectively inhibits bacterial protein synthesis through interaction with prokaryotic ribosomes, while it has no effect on eukaryotic protein synthesis [5]. For this unique mechanism of action, pleuromutilin had rarely cross-resistance to the marketed antibacterial drugs and thus received more and more attention from medicinal chemists to develop new antibacterial drugs.

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This review mainly focuses on the mechanism of action and structure-activity relationship of pleuromutilin derivatives. Additionally, methods of preparing pleuromutilin derivatives and the future prospects of pleuromutilin-derived compounds in antibacterial research are also discussed.

PLEUROMUTILIN DERIVATIVES

The chemical modification of pleuromutilin focused on variations of the C-14 acyloxy group [6, 7]. From this research, tiamulin (**2**, Fig. **1**) was successfully developed as one of the oral antibiotics for veterinary use [8, 9]. In order to improve the tiamulin potency, researchers in Sandoz modified the ester side-chain on C-14 of tiamulin and led to a second veterinary agent, valnemulin (**3**, Fig. **1**) [10]. Valnemulin is the first veterinary medicinal premix which has been approved across the EU and categorized as the only prescription medicine [11]. Though tiamulin and valnemulin have been successfully employed for veterinary use, most of other semi-synthetic pleuromutilins are scarely used. Because they are rapidly and extensively metabolized by cytochrome P450 metabolism *in vivo*, which limits their oral bioavailability [12]. Thus, development in this class of compounds has been focused on chemical modifications and structure-activity studies for exploiting new antibiotic use in humans. In 1982, Berner *et al.* synthesized azamulin (**4**, Fig. **1**) and evaluated its antibacterial activity in *in vitro* [13]. Although azamulin had entered phase I clinical studies in volunteers, it failed to be used in humans for its rapid metabolism and subsequent excretion [14]. Subsequently, medicinal chemists at GlaxoSmithKline identified retapamulin (**5**, Fig. **1**), which was a new pleuromutilin analog with excellent *in vitro* antibacterial activity. Retapamulin was approved as a topical antimicrobial agent for the treatment of human skin infections in 2007 by Food

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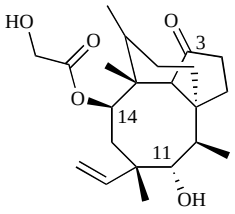
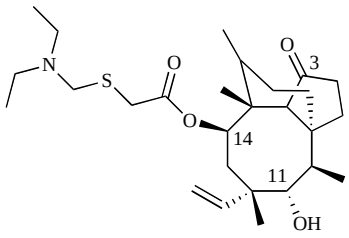


1. Pleuromutilin



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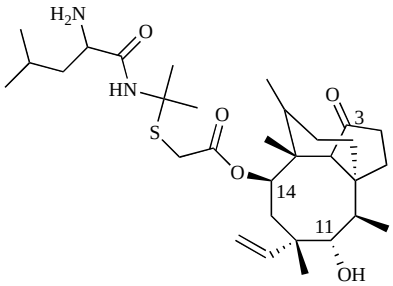
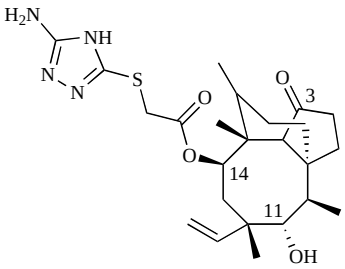
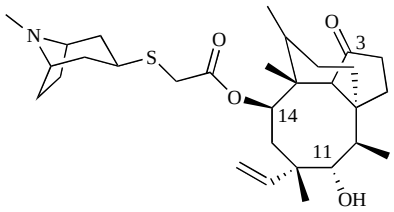


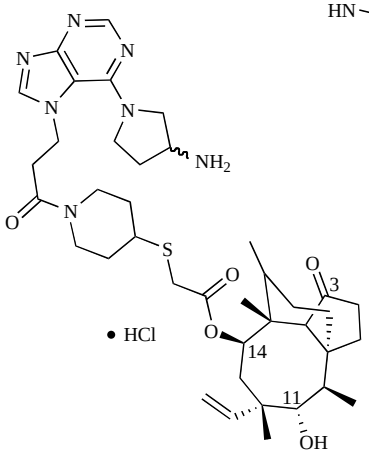
3. Valnemulin



4. Azamulin



5. Retapamulin



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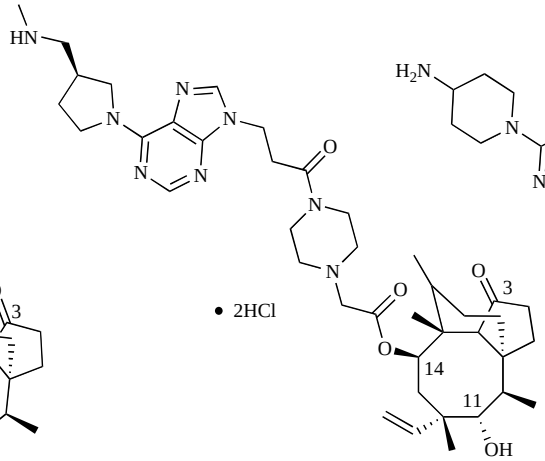

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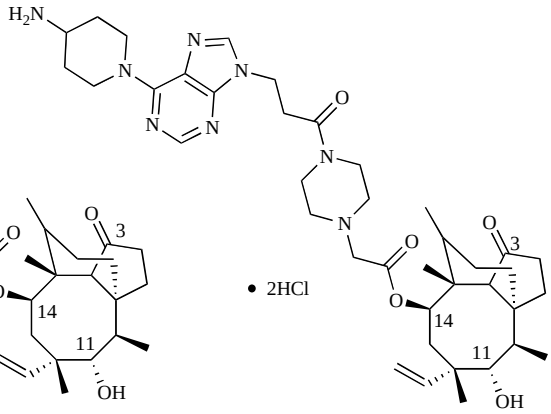

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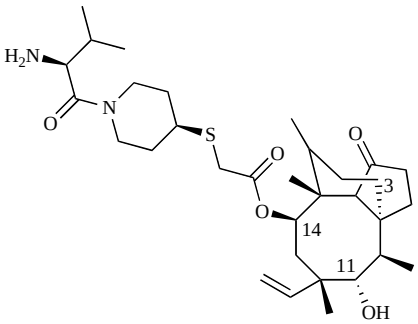
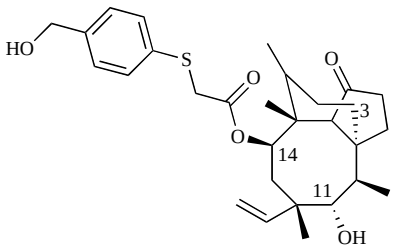


9. BC-3205



10. BC-7013

Fig. (1). Chemical structures of compounds **1~10**.

and Drug Administration (FDA) [14]. The success of retapamulin makes the medicinal chemists pay more attention to pleuromutilin. Recently, a series of water-soluble pleuromutilin analogues with a purine ring has been reported by Hirokawa and coworkers [13-16]. Among these derivatives, **6**, **7**, and **8** (Fig. **1**) were found to exhibit strong *in vitro* and vivo antibacterial activities [14]. Additionally, the method to prepare the pleuromutilin framework has been reassessed by researchers [17]. Two new pleuromutilin derivatives, BC-3205 (**9**, Fig. **1**) and BC-7013 (**10**, Fig. **1**), have entered into clinical trails [18].

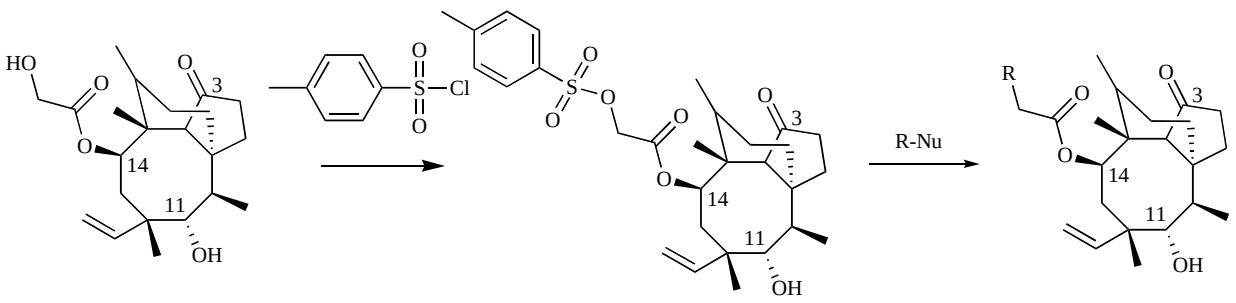
Due to their unusual tricyclic structure, pleuromutilin and its derivatives have attracted considerable attention of synthetic researchers. An elegant total synthesis of pleuromutilin has been reported by Gibbons for the first time in 1982[19]. This synthetic approach was based on a sequential Michael strategy. In 1989, Boeckman and coworkers [20] reported a novel approach to the construction of tricyclic framework of pleuromutilin. In that new synthetic route, readily available materials were used to synthesize pleuromutilin in 25 steps. Recently, two new synthetic studies on pleuromutilins' scaffold were reported by Liu *et al.* [21] and Findley *et al.* [22], respectively. Although the approach to the skeleton of pleuromutilin has been optimized, the modification of the side chain at C14 of pleuromutilin might be the most promising approach to obtain diverse pleuromutilin derivatives. One of the synthetic route which can obtain pleuromutilin derivatives *via* modifying the C14 of pleuromutilin is shown in Scheme **1**.

STRUCTURE-ACTIVITY RELATIONSHIPS

The chemical modification of pleuromutilin had been made for investigating its structure-activity profile and improving its potency after the structure of pleuromutilin was identified by Kavanagh *et al.* [6, 7, 23, 24]. Egger and Reinshagen demonstrated for the first time that chemical modification of the C14 side chain of pleuromutilin could optimize its activity against bacteria and solubility in water [7]. Their studies indicated that antibacterial activity was largely determined by the substituent group at C14 in pleuromutilin derivatives [6, 7, 25]. Further studies showed that the pleuromutilin derivatives with a thio-ether substituent at their C14 side-chain were extremely active [8]. These studies led to the appearances of tiamulin (**2**, Fig. **1**) and valnemulin (**3**, Fig. **1**), which were both this type of pleuromutilin derivatives, as new antimicrobial agents for veterinary use [10].

To find a pleuromutilin derivative for human use, more medicinal chemists have described their efforts in the modification of the C14 side chain of pleuromutilin. In order to improve the bioavailability of pleuromutilin, Berner and coworkers had synthesized several series of pleuromutilin derivatives with different substituent since 1980 [26-35]. From these researches, azamulin (**4**, Fig. **1**), one azole derivative of pleuromutilin with a thio-ether substituent at the C14 side-chain, was discovered and progressed as far as studies in volunteers. Unfortunately, azamulin was not successfully developed as a drug [12]. However, its good antibacterial activity in *in vitro* makes medicinal chemists develop pleuromutilin derivatives with a cyclic or bicyclic tertiary amine moiety into their thio-ether substituent. The most successful example from this series of pleuromutilin derivatives was retapamulin (**5**, Fig. **1**), which was the first antibacterial drug known as pleuromutilin derivative to be approved for use in humans [36]. It has been used for the topical treatment of skin structure infections (SSSIs), impetigo, infected small lacerations, abrasions or sutured wounds. In addition to retapamulin, this type of pleuromutilin derivatives, including BC-3205 (**9**, Fig. **1**), BC-7013 (**10**, Fig. **1**), BC-3004 (**11**, Fig. **2**), BC-3080 (**12**, Fig. **2**) and BC-3291 (**13**, Fig. **2**) also had good antibacterial activity [18]. Among these derivatives, BC-3205 (**9**, Fig. **1**) and BC-7013 (**10**, Fig. **1**) have entered Phase I clinical studies in volunteers [37, 38]. So it is possible to get a new antibiotic from this series of derivatives.

Except for the above 14-ester derivatives, there are other two types of pleuromutilin derivatives, 14-carbamate derivatives and the derivatives having a purine ring, which have attracted attention from medicinal chemists. Like the 14-ester derivatives of pleuromutilin, 14-carbamate derivatives usually have good, broad-spectrum activity. The 14-carbamate derivatives show the best potency and most balanced antibacterial spectrum for their acyl-carbamates [8]. The phenol SB-225586 (**14**, Fig. **2**) and its methyl ether SB-222734 (**15**, Fig. **2**) which have excellent antibacterial potency belong to this series. However, both of them did not progress further due to their low solubility in water. By introducing a basic amino group into the C14 side-chain of 14-carbamate derivatives, medicinal chemists improved their solubility and then got SB-247386 (**16**, Fig. **2**) which had potent, broad-spectrum antibacterial activity [8]. Another example of this series of pleuromutilin derivatives was SB-264128 (**17**, Fig. **2**), which had excellent antimicrobial properties and good bioavailability in rodents. It also had good oral bioavailability in dogs, but it was proved to be



Scheme 1. One of the synthetic route of pleutromutilin derivatives.

unsuitable for further development for its myocardial effects in rats [8]. Luckily, the myocardial effects shown by SB-264128 (**17**, Fig. **2**) have been proved not to be a pleuromutilin class effect. In spite of these derivatives, many of other 14-carbamate derivatives of pleuromutilin also had good antibacterial activities. Compound **18** (**18**, Fig. **2**) was stated to show good activity (minimum inhibitory concentration, MIC≤4μg/ml) against *S. aureus*, *S. pneumoniae* and *M. catarrbalis* [12]. Furthermore, compound **18** also showed good activity against *H. influenzae* and *C. pneumoniae*, but no specific biological data were presented. In WO06070671, another analogue of this type of pleuromutilin derivatives, compound **19** (**19**, Fig. **2**) (MIC=0.5μg/ml) was stated to show lower MIC value than tiamulin (MIC=1~2μg/ml) against *S. pneumoniae* IID 553. Another patent from SmithKline Beecham (SKB) claimed that compound **20** (**20**, Fig. **2**) could be used to protect the infections against some drug-resistant bacterium [12].

For developing metabolic stable pleuromutilin derivatives with good antibacterial activity which would be better than previous analogs, a series of pleuromutilin derivatives bearing a purine ring had been prepared by Kinoshita and coworkers [13-16]. Based on the development of compound **6**, they found that compounds **21** and **22**, Fig. (**3**), which had good solubility in water and good pharmacokinetics, showed excellent *in vitro* and *in vivo* antibacterial activity against a number of Gram-positive pathogens when compared to azamulin and vancomycin [15]. In order to optimize compounds **6**, **21** and **22**, Kinoshita *et al.* designed and synthesized much more pleuromutilin derivatives with the same purine ring. From these novel pleuromutilin derivatives, compounds **23** and **24**, Fig. (**3**), have good solubility in water, promising *in vitro* antibacterial activity against various Gram-positive bacteria and potent *in vivo* efficacy [16]. Chemical modification of compound **24** resulted in the discovery of pleuromutilin derivatives, which had not only a purine ring but also a piperazine ring spacer [14]. Structure-activity relationship (SAR) of these derivatives showed that all of them had potent *in vitro* antibacterial activity with just slight differences. Among all of these derivatives, compounds **7** and **8** not only exhibited excellent effect *in vitro* antibacterial

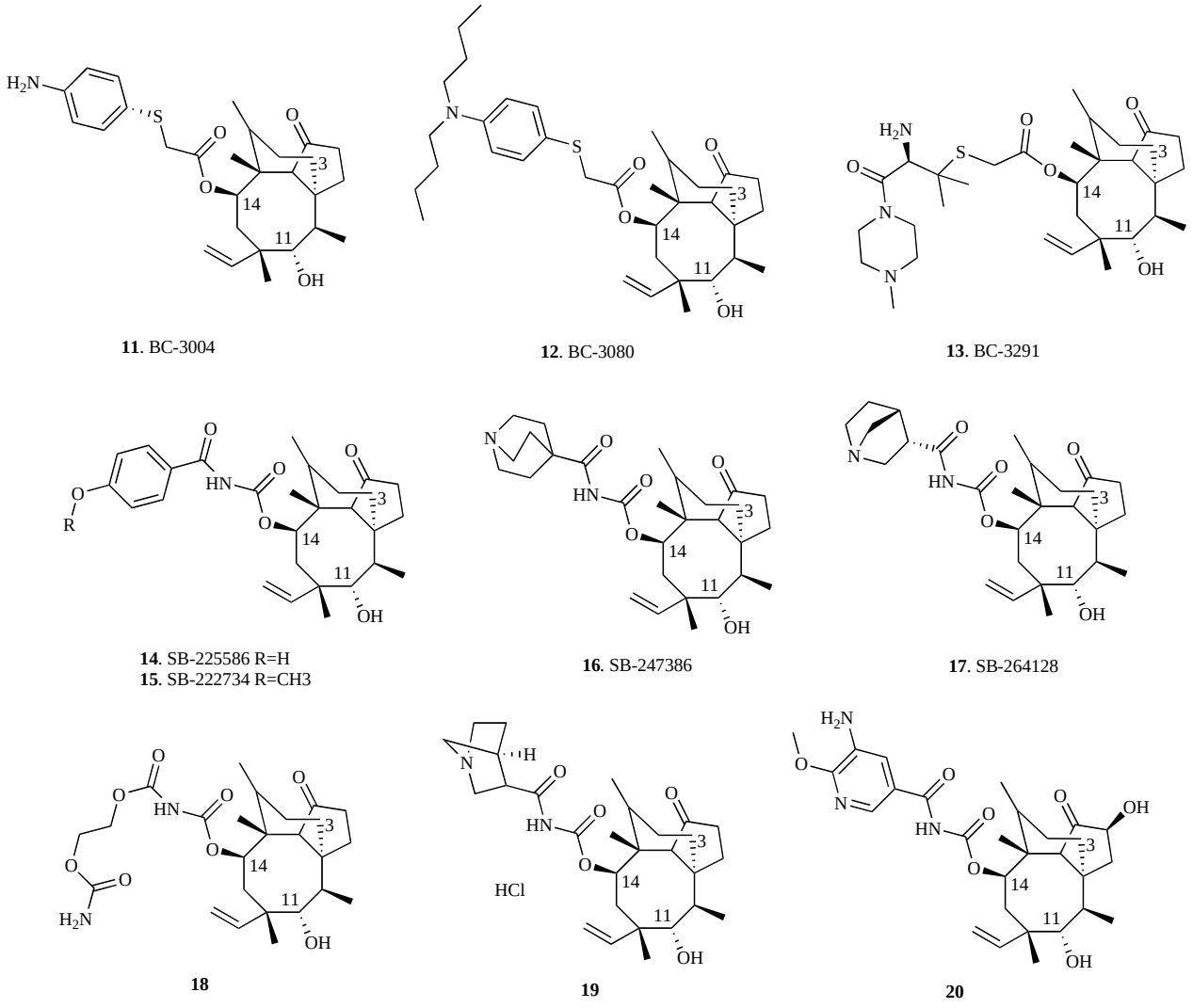
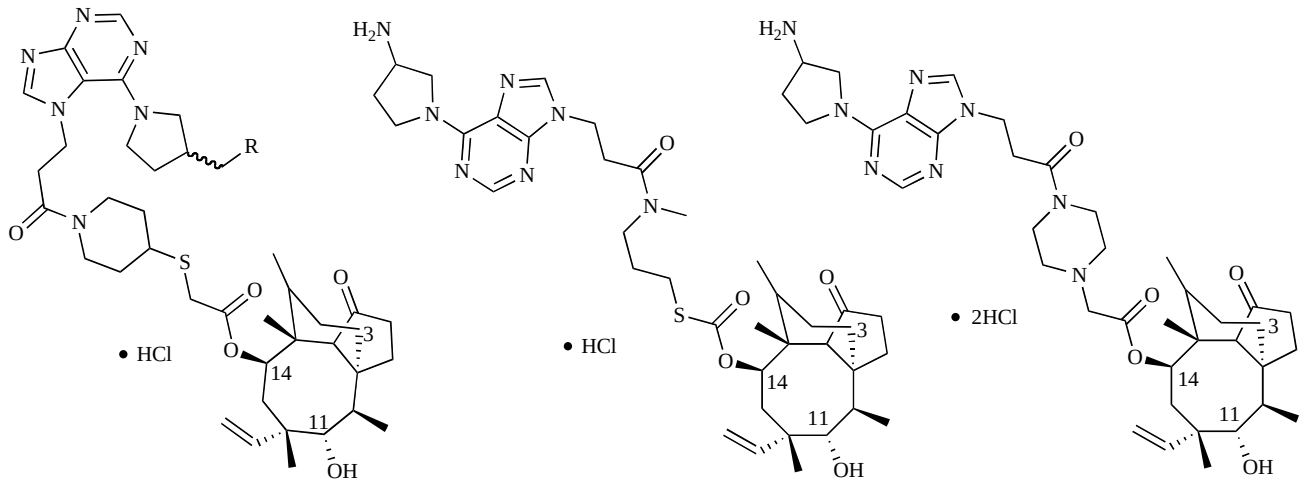


Fig. (2). Chemical structures of compounds **11~20**.



21. R=NH_2
22. R=$NHCH_3$

23

24

Fig. (3). Chemical structures of compounds **21~24**.

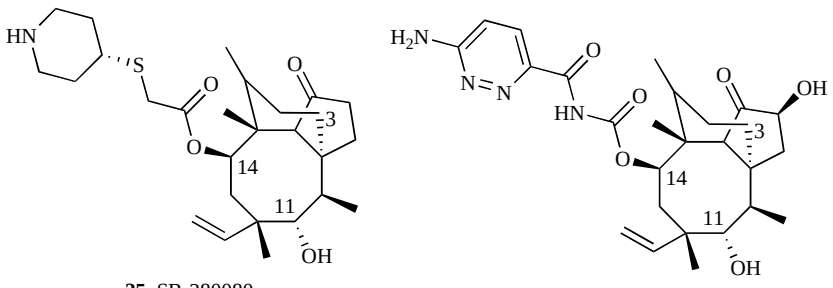
activity but also potent *in vivo* efficacy. The results from Hirokawa and coworkers also indicated that introduction of methyl group or ethyl group into the piperazine ring spacer of compound **24** might cause a slight increase in *in vitro* antibacterial activity or significantly decrease the *in vivo* efficacy [14]. *In vivo* efficacy of pleuromutilin derivatives could be improved while the thio-ether type side chains of pleuromutilin derivatives were changed into piperazine ring spacer. So it was believed that *in vivo* efficacy of pleuromutilin derivatives with a purine ring might be improved through changing their central spacer. And the research about changing central spacer of this type of pleuromutilin derivatives might lead to another novel series of pleuromutilin derivatives which have both excellent effect in *in vitro* antibacterial activity and potent *in vivo* efficacy.

MECHANISM OF ACTION

The early work on the mode of action of tiamulin and pleuromutilin had been comprehensively reviewed by Hogenauer in 1979 [39]. In 1974, Hogenauer and colleagues demonstrated that pleuromutilin derivatives selectively inhibited bacterial protein synthesis through interaction with substrate binding at the acceptor and donor site (A- and P-site, respectively) of the ribosome. And this inhibition had no effect on eukaryotic protein synthesis and did not bind to mammalian ribosomes [40]. Accordingly, subsequent experiments using equilibrium dialysis techniques demonstrated that pleuromutilin derivatives bound specifically to one site per ribosome, and the binding to 70S ribosomes was tight yet reversible [41]. But the follow-up studies with labeled tiamulin suggested that 2 molecules bound to each 70S ribosome [42]. On the other hand, it had been reported that the presence of pleuromutilin derivatives would not interfere with protein synthesis after peptide-chain elongation began [43].

In 2001, footprinting analysis was used by Poulsen and coworkers to study how the bacterial protein synthesis was inhibited by pleuromutilin derivatives [44]. Their study confirmed the research of Hogenauer *et al.* [41] and demonstrated that tiamulin and valnemulin were strong inhibitors of peptidyl transferase. The inhibitions of tiamulin and valnemulin were due to their interaction with domain V of 23S ribosomal RNA (rRNA) at nucleotides A2058-9, U2506 and U2584-5. This study also showed that tiamulin and valnemulin could bind concurrently with the macrolide erythromycin but competed with the macrolide carbomycin, which was a peptidyl transferase inhibitor. Poulsen and coworkers' footprinting results were confirmed by crystallography data from Schluenzen *et al*. in 2004[9].

Schluenzen *et al.* presented crystal structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with the tiamulin. Their results showed that tiamulin bound to the peptidyl transferase center (PTC) of the 50S ribosomal subunit with its tricyclic nucleus located inside a cavity confined by residues G2061, A2451, C2452, A2503, U2504, G2505, U2506. The binding site of tiamulin overlapped that of both A- and P-site tRNA substrates and thus explained its direct inhibition of peptide bond formation [9]. This study led to the discovery of mechanism for pleuromutilins' activity. Afterward, Davidovich *et al.* provided the crystal structures of complexes of the 50S ribosomal with each of other three semisynthetic pleuromutilins, SB-275833 (retapamulin, **5**, Fig. **1**), SB280080 (**25**, Fig. **4**), and SB-571519 (**26**, Fig. **4**) [45]. Their results confirmed that the C11 hydroxyl groups of these three pleuromutilins were located in a position suitable for hydrogen bonding to G2505 phosphate, which was the same as tiamulin[45]. The electron density maps of SB-571519 (**26**, Fig. **4**) complexes showed that the C-2 hydroxyl group of SB-571519 might be involved in polar interaction or an H-bond with O3' or O5' phosphoester of G2505. Additionally, the C-21 keto group of these pleuromutilins was found to interact with G2061 *via* two or three H-bonds, while the C14 extension of which seemed to be involved in only minor hydrophobic contact with ribosomal nucleotides [45]. With binding to the pleuromutilins, the nucleotide



25. SB-280080 **26**. SB-571519

Fig. (4). Chemical structures of compounds 25 and 26.

U2585 shifted away from its native conformation to avoid steric hindrance [45-47]. The interaction between pleuromutilins and U2582 could stabilize the conformations of both U2585 and U2506 in the bound state. It has been revealed that U2585 could hinder the synthesis of 50S subunits after it acquires a non-productive orientation [45, 48, 49].

Then the detailed mode of action of retapamulin with bacterial ribosomes has been confirmed by Yan and coworkers [50]. Their data suggested that retapamulin inhibited P-site substrate binding independently of substrate concentration and consistently with an antagonistic. Pleuromutilin derivatives protected the peptidyl transferase rRNA based on U2584 and U2585, which implicated in the binding of tRNA to the P-site [18, 51]. It was reasonable to conclude that pleuromutilin derivatives could inhibit the synthesis of 50S subunits through interacting with U2584 and U2585. The protective effect of pleuromutilin derivatives at U2584 and U2585 was due to their C14 side-chain extension [18]. This finding was of particular importance. Long *et al.* demonstrated that pleuromutilin drugs with enhanced antimicrobial activity might be obtained by maximizing the number of interactions between the side chain moiety and the peptidyl transferase cavity [52].

METABOLISM AND *IN VIVO* EFFICACY

Semi-synthetic pleuromutilins suffer from rapid and extensive cytochrome P450 metabolism *in vivo*, which limits their oral bioavailability as well as embraces their development for oral treatment of bacterial infections [53]. Early studies with the azole derivative azamulin had shown that the (2R)- and (8R)-hydroxy derivatives, which had no antibacterial activity, were the major metabolites produced in *in vivo* [8].

Tiamulin is rapidly metabolized in *in vivo* through hydroxylating at the C-2 and C-8 positions of its tricyclic nucleus by cytochrome P450 [54]. Laber and Schutze had evaluated the *in vivo* efficacy of tiamulin in chickens and turkeys which had been infected by *Mycoplasma* strains [55]. Their studies were confirmed by other researchers [56, 57]. The results showed that tiamulin had a superior efficacy in both prophylactical and therapeutical tests. Additionally, the studies also demonstrated that the tiamulin could be used for treatment of mycoplasmosis in chickens and turkeys in a dosage of 0.025% and in a dosage of 0.0125% for prophylaxis in flocks at risk respectively. In 2002, the influence of tiamulin treatment on experimental avian intestinal spirochetosis was evaluated by Hampson and coworkers [58, 59]. The results showed that tiamulin was highly effective when used for the treatment of avian intestinal spirochetosis in broiler breeders and layer hens at 25 mg/kg body weight per day over 5 days in artificial infection studies with *Brachyspira pilosicoli* and *Brachyspira intermedia*, respectively. These studies were confirmed by Burch *et al*. in a field case of avian intestinal spirochetosis caused by *Brachyspira pilosicoli* in laying hens but at 12.5 mg/kg for 3 days [60].

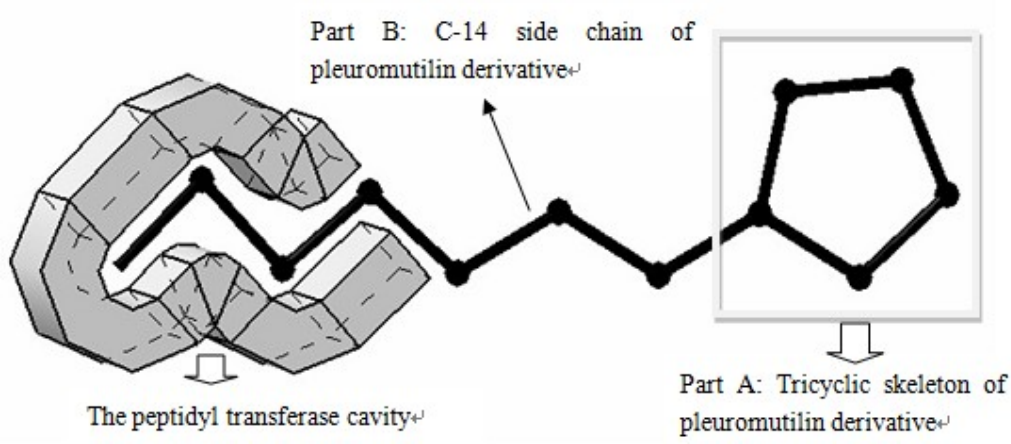
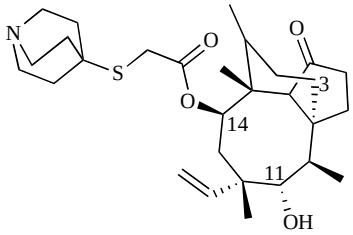


Fig. (5). Mode of action of the interaction between C-14 side chain of pleuromutilin derivative and the peptidyl transferase cavity.

Compound **27**, which had a C-14 sulfanyl-acetate side-chain, tends to be metabolized more quickly then compounds **18** and **19** [8]. It seemed that there was a much better balance between antimicrobial activity and metabolic stability for 14-carbamate derivatives, such as compounds **18** and **19**. Actually, the oral bioavailability of compound **19** was 63% in rats, 60-90% in dogs and 22% in cynomolgus monkeys [61]. Additionally, compound **19** also had excellent antimicrobial properties in animal infection models through oral administration [62]. In addition to the C14 side chain, chemical modification on the 5- and 6-membered rings of pleuromutilin derivatives could also improve their metabolic stability [30, 32, 33]. In their study, Berner and coworkers found that inversion of the stereochemistry at C6 of pleuromutilin derivatives could improve their metabolic stability at the expense of antimicrobial efficacy [30]. Furthermore, it had been found that slightly modified conformation at C1, C2 and C8 also resulted in improved metabolic stability of pleuromutilin derivatives [32, 33].



27. SB268091

Fig. (6) Chemical structures of compound **27**.

Although retapamulin has been used as a topical antimicrobial agent for the treatment of human skin infections, research about its metabolism is rarely. Retapamulin possessed equivalent or superior antibacterial activity to other commonly used antimicrobial agents against multitude clinical resistant strains which had been isolated from skin and skin structure infections in many *in vitro* studies. In an *in vivo* study, Singley and coworkers tested the efficacy of retapamulin against resistant strains of *Staphylococcus aureus* in a murine wound infection model [63]. For F306 (methicilin- and azithromycin-resistant) and X32717 (methicilin-, mupirocin-, azithromycin- and levofloxacin-resistant), the retapamulin MIC values were both 0.12 μg/ml. When tested against T63256 (methicillin-, mupirocin-, azithromycin- and levofloxacin-resistant), retapamulin yielded MIC values of 0.06 μg/ml. Against 1080 (mupirocin- and azithromycin-resistant) and S5112 (mupirocin-, azithromycin- and levofloxacin-resistant), the retapamulin MIC values were 0.06 and 0.03 μg/ml, respectively. Additionally, compared to other common antibiotics, retapamulin demonstrated superior efficacy in reducing bacterial counts in that infection model [64].

CONCLUSIONS

The pleuromutilin derivatives were a series of antibiotics that had been used in veterinary medicine. Their antibacterial spectrums, which included SSSI pathogens and infections of the respiratory tract, rendered them attractive candidates for researching and developing drug for human use. The success of retapamulin, which was used as a topical antibiotic for clinical use in humans, spurred the interest of medicinal chemists for discovering, exploiting and developing novel pleuromutilin derivatives for human use. Structure-activity relationship studies of pleuromutilin derivatives mainly focused on the modification of their C14 glycolic acid side chain and thus a number of derivatives were successfully synthesized and developed [11]. Among these derivatives, there was a class of analogues bearing a purine ring which had excellent antibacterial activity, good solubility in water, good pharmacokinetics and ADME properties, and improved metabolic stability. All these activities of this type of pleuromutilin derivatives were better than other ones which had been synthesized before [11]. Despite all of these derivatives, more and more effective pleuromutilin derivatives are necessary to be developed to treat infections which have been infected by resistant bacterial strains. In this regard, the detailed information about how tiamulin targeted the peptidyl transferase center of 50S ribosome would be useful in the rational design of new pleuromutilin derivatives [52]. Additionally, a click chemistry approach to pleuromutilin conjugated with different nucleoside fragments as side chain extensions had been synthesized to promote rational design of pleuromutilin based drugs [65]. Although the efficacy of most of these derivatives in *in vitro* has been reported, further studies are needed to establish the clinical usefulness of them. Actually, except for the retapamulin, no full clinical data are available on other pleuromutilin derivatives. Thus, the challenging step of developing new antibiotic from pleuromutilin derivatives will be the success of clinical trials of promising derivatives and their introduction into the clinic [12].

Accompanying with the application of computer-aided drug design and other modern lead-optimization approaches to the semi-synthesis of new pleuromutilin derivatives, more and more members of this potentially exciting and clinically valuable class of compounds have entered clinical trials for systemic therapy. We believe that the development of pleuromutilin derivatives might give derivatives with improved safety profiles and then yield candidates suitable for development as treating community-acquired infections in the future.

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