Amphotericin B: From Derivatives to Covalent Targeted Conjugates

Miloš Sedlák*

Department of Organic Chemistry & Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, CZ-532 10 Pardubice, The Czech Republic

Abstract: This mini-review summarizes the development of the derivatives and conjugates of Amphotericin B (AMB) which have not been clinically applied so far but are therapeutically promising. Effects of chemical modifications of AMB upon biological activities of the resulting derivatives are discussed. The examples presented include variation of functional groups in AMB, covalent conjugates with other molecules, poly(ethylene glycols) and polysaccharides. Possibilities of targeted delivery of AMB are discussed.

Key Words*:* Amphotericin B, antimycotics, systemic mycosis, poly(ethylene glycol), polysaccharides, drug targeting, pH-sensitivity, β-glucosidase-sensitivity.

Dedicated to Professor Vladimír Macháček on the occasion of his 65th birthday.

1. INTRODUCTION

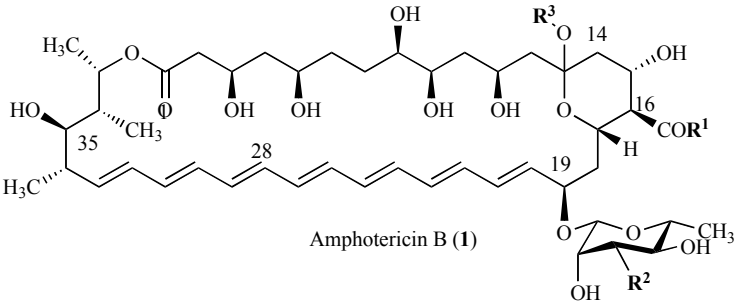
Amphotericin B (AMB) (**1**) Fig. (**1**) is a polyene macrocyclic, membrane-active antifungal antibiotic drug, which was isolated in the second half of the 20th century from *Streptomyces nodosus* [1]. Absolute configuration of AMB was determined in 1970 by means of the single-crystal analysis of the *N*-iodoacetyl derivative of AMB [2]. The main structural motifs of AMB molecule are a 38-membered cyclic lactone, which is composed of a polyene moiety and a part carrying hydroxyl groups; this basic macrocycle is further attached to the mycosamine molecule [3]. It should be emphasized that AMB still attracts attention of many research teams for many reasons [4]. For example AMB belongs among efficient life-saving drugs in treatment of systemic fungal infections [5], which occur in immunosuppressive patients, e.g., after organ transplantations, in the cases of Acquired Immunodeficiency Syndrome (AIDS), and in patients suffering from tumors and hematological malignity. However, the mechanism of its biological action is not fully understood yet [4]. It is known that AMB action is based on the selective interaction with ergosterol. Ergosterol, which is located in plasmatic membranes of fungi, forms with AMB barrel-stave channels which disrupt their barrier function. The channels formed allow escaping of K^+ ions and/or some small molecules, which eventually causes death of the fungal cell [6-9]. Clinical applications of AMB are particularly limited by its poor solubility in water and also by its potential organ toxicity, such as nephrotoxicity, which is explained by interaction of AMB with the cholesterol molecules present in membranes of mammalian cells [10-11]. In order to increase the therapeutic index of AMB, several pharmaceutical dosage forms are being used which enable solubilizing in aqueous medium. The oldest formulation designed for intravenous administration is a micellar suspension of deoxycholic acid and AMB (Fungizone) [12]. In recent years new drug delivery systems such as liposomes, nanospheres (polymeric micelles) and microspheres have been also used for the formulations of AMB [13-16]. Among clinically adopted current pharmaceutical dosage forms of AMB belong non-covalent lipid complexes (ABLC) [17, 18] and liposomal forms (AmBisome) [19], which are colloidal systems composed of bio-degradable matrices and AMB. These formulations ensure smoother release of AMB accompanied by its restricted distribution in the kidney, thereby lowering its nephrotoxicity [20]. In the case of the above-mentioned complexes, a significant increase in antifungal activity *in vitro* was observed [21]. On the other hand there exist a number of studies that have not shown an increase of the *in vitro* MICs of lipid AMB formulations [22]. The aim of this review is to summarize and discuss the existing findings concerning only covalent derivatives and conjugates of AMB with other molecules and polymeric carriers, to summarize the development in this area, and to draw attention to derivatives and conjugates that have not been therapeutically applied yet, but are promising and can represent a more efficient way of treatment of systemic fungal infections.

*Address correspondence to this author at the Department of Organic Chemistry & Technology, Faculty of Chemical Technology, University of Pardubice, Studentská 573, CZ- 532 10 Pardubice, The Czech Republic; Fax: +420 466 037 068; E-mail: Milos.Sedlak@upce.cz

2. DERIVATIVES OF AMB

The isolation of AMB (**1**) Fig. (**1**) (13-(*R*)), was followed by syntheses of its derivatives focused on increasing its therapeutic index or obtaining model substrates designed for studies of interactions with sterols cell membranes Figs. (**1**-**3**). One of the earliest derivatives prepared was the methyl ester of AMB (**2**) [23, 24], which besides antifungal activity exhibits also inhibition activity towards HIV-1 [25]. In contrast to that, the methyl acetal **3** Fig. (**1**) (13-(*S*)), which is formed during heating of methanolic solution of AMB (60 °C, 12 hours), represents an undesirable impurity occurring during isolation of AMB. This side product possesses half the antifungal activity (MIC_{50} = 1μg/ml) against *Candida*

1389-5575/09 $55.00+.00



Amphotericin B (**1**)

1: R^1= OH, R^2= NH_2, R^3= H;

2: R^1= OCH_3, R^2= NH_2, R^3= H;

3: R^1= OH, R^2= NH_2, R^3= CH_3 ;

4: R^1= OH, R^2= NH-$CH_2C_6H_4$-4-X, X = H, Cl, Br, I, OH, NO_2, $N(CH_3)_2$, OC_2H_5, $CH_3C_2H_5$,R^3= H;

5: R^1= OH, R^2= $NHCOCH_2X$, X = H, Cl,$(CH_2)_2CO_2H$,$(CH_2)_2CO_2CH_3$, R^3= H;

6 R^1= $NH(CH_2)_2CH_3$, R^2= NH_2, R^3= H;

7: R^1= $NH(CH_2)N^+H(CH_3)_2$, R^2= NH_2, R^3= H;

8: R^1= OCH_3, R^2= $N^+(CH_3)_3$, R^3= H;

9: R^1= OH, R^2= $N(COCH_2N(CH_3)_2)_2$, R^3= H;

10: R^1= OCH_3, R^2= $N(COCH_2N(CH_3)_2)_2$, R^3= H;

11: R^1= OH, R^2= $N(COCH(CH_3)N(CH_3)_2)_2$, R^3= H;

12: R^1= OCH_3, R^2= $N(COCH(CH_3)N(CH_3)_2)_2$, R^3= H;

13: R^1= OH, R^2= $N((CH_2)_3NH_2)_2$,R^3= H;

14: R^1= OH, R^2= $N((CH_2)_3CO_2H)_2$, R^3= H;

15: R^1= $NH(CH_2CH_2O)_6CH_3$, R^2= NH_2, R^3= H;

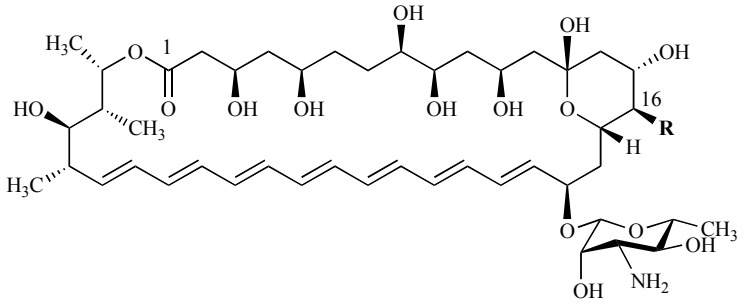
16: R^1= X, X = CH_3, C_6H_5, pyrrol-2-yl, $(CH_2)_2CH{=}CH_2$, R^2= NH_2, R^3= H.

Fig. (1). Structure of AMB (**1**) and its derivatives **2-16**.

albicans as compared with AMB itself [26]. Further modifications were performed using targeted synthesis modifying the amino group or the carboxyl group of AMB. Reductive amination of AMB (with 4-X-C_6H_4-CHO/Na(CN)BH_3) gave substituted *N*-benzyl derivatives **4** Fig. (**1**), which exhibit antifungal activities comparable with that of AMB, but are three times less toxic for mice [27]. The acylation of amino group and amidation or esterification of carboxyl group gave products **5-12**, which possess lower biological activities than free AMB [28-30]. Comparison of biological activities of the derivatives prepared (**5-12**), and the studies of their interactions with sterols showed that the positively charged nitrogen atom of amino group in AMB is necessary for effective interaction with ergosterol. However, it is still not clear what is the role of free carboxyl group. The findings [31] about interactions of yeast membranes with exogenous polyamines led to synthesis of *N,N*-di-(3-aminopropyl)-AMB (**13**), which exhibits fifteen times higher inhibition activity (MIC = 0.018 μg/ml) against *Saccharomyces cerevisae* as compared with free AMB (MIC = 0.28 μg/ml) [32, 33]. On the other hand, *N,N*-di-(4-butanoic acid)-AMB (**14**) lost its antifungal activity (MIC > 10 μg/ml) [33]. Introduction of hexaethylene glycol fragment on nitrogen atom of amide-AMB gave a water-soluble analog **15**. The *in vitro* tests of five isolated *Candida albicans* showed **15** to have potency similar to that of AMB [34]. Derivative **15** was administered to a mice infected intravenously with *Candida albicans*. The *in vivo* tests showed that compound **15** was well tolerated up to 30 mg/kg of body weight per dose, an amount that would be lethal with AMB. Later on, these authors examined [35, 36] the efficiency of **15** against cutaneous infection with *Leishmania amazonenzis* and visceral infection with *Leishmania donovani* in susceptible BALB/c mice. The cutaneous lesions showed a remarkable response to therapy with **15** at the dose of 30 mg/kg body weight per day. At this dose (1-5 mg/kg/day), the efficiency of **15** was equivalent to, or better than AMB. Mice infected intravenously with 10^7 *Leishmania donovani* promastigotes and treated with **15** showed 4-log reduction in the parasite burden in the liver and spleen compared to untreated mice. Further semi-synthetic derivatives of AMB that were prepared included ketones **16** [37]; however, their biological activities have not been completely evaluated yet [38].

Furthermore, the same authors published the preparation of (14*R*)-hydroxy-AMB methyl ester and its (14*S*)-epimer [39] and later also C-16 oximino and vinyl AMB derivatives **17** [40] Fig. (**2**). These derivatives were prepared by the reaction of a suitably protected AMB C-16 aldehyde with hydroxylamine derivatives and Wittig reagents, respectively, followed by sequential removal of the protecting groups. Compounds **17** possesses a potent antifungal activity *in vitro*, similar or in some cases superior (R : (*E*)-CH=N–OCH_3; MIC = 0.5 μg/ml; R = –$CH{=}CH_2$; MIC = 2 μg/ml *Candida albicans,*) to that of AMB itself (MIC = 4 μg/ml). With the exception of the C-16 (*Z*)-methoxime AMB (EH_{50} = 2.7 μg/ml), the other derivatives do not show significantly reduced hemolytic activity against mammalian erythrocytes compared with AMB (EH_{50} = 3.2 μg/ml).

A series of amide derivatives **18-21** Fig. (**3**) with heterocyclic fragments was prepared for the purpose of systematic studies of interactions with lipid membranes [30, 41, 42].



17: R= CHO; (*E*)-CH=NOCH$_3$; (*Z*)-CH=NOCH$_3$;(*E*)-CH=NOH;(*Z*)-CH=NOH; CH=CH$_2$; (*E*)-CH=CH-CO$_2$CH$_3$;(*E*)-CH=CH-CO$_2$H;(*E*)-CH=CH-CH$_2$OH; (*E*)-CH=CH-CO$_2$CH$_2$CH=CH.

Fig. (2). Structure of C-16 oximino and vinyl AMB derivatives **17**.

Molecular dynamics simulation shows that *N*-(1-piperidinpropionyl)-AMB methyl ester (**18**) and *N*-(*N'*-3-dimethylaminopropylsuccinimido)-AMB methyl ester (**19**) penetrate deeper into hydrophobic region of lipid membrane and also exhibit lower toxicity as compared with AMB [41]. Low toxicity and biological activity comparable with that of AMB was found not only for derivatives **18-20,** but also for methyl ester of *N*-methyl-*N*-D-fructosyl AMB **21** Fig. (**3**) and the corresponding free acid [30, 42].

Covalent conjugates **22-26** [43] and **27** [44] Fig. (**4**) were tested for their effects on penetration of K^+ ions through phospholipide membranes. Besides the cholesterol conjugate **25** [43], also the conjugate with ergosterol was prepared, and its arrangement in the solid phase was studied by NMR and CD spectra [45]. Covalent dimers of AMB were also prepared [46] with linkages of different lengths between the amino group of one AMB molecule and carboxyl group of the other (–NH–CO–, –NH–CH$_2$–CH$_2$–CO–, –NH–CH$_2$CO–NH–(CH$_2$)$_3$–CO–). However, the dimers show more than five times lower activity against *Aspergillus niger* as compared with free AMB. The fluoro derivatives 14-F-AMB and 28-^{19}F-AMB were synthesized to study the ion-channel formation mechanisms; they exhibited antifungal activities similar to those of AMB itself [47]. The AMB derivatives that can be prepared by total synthesis involve not only the non-polyene analog of AMB [48] but also 35-deoxy AMB [49, 50].

Supramolecular systems are represented by the conjugates with substituted calix[4]arenes **28**, **29** binding four molecules of AMB Fig. (**5**) [51]. These supramolecules adopt cone conformation that mimics the structure of a membrane pore. The antifungal activity of the conjugates was superior or similar to the free AMB. Furthermore, the hemotoxicity of these conjugates was considerably lower than that of monomeric AMB. The formation of ion channels in the lipid bilayer by the AMB-calix[4]arenes was studied by measuring the K^+ efflux from various liposomes [51].

Further studies include supramolecular conjugates **30** with carbon nanotubes Fig. (**6**) [52-54]. These studies reveal that AMB covalently linked to the carbon nanotubes is taken up by mammalian cells without presenting any specific toxic effect. Furthermore, AMB bound to the carbon nanotubes

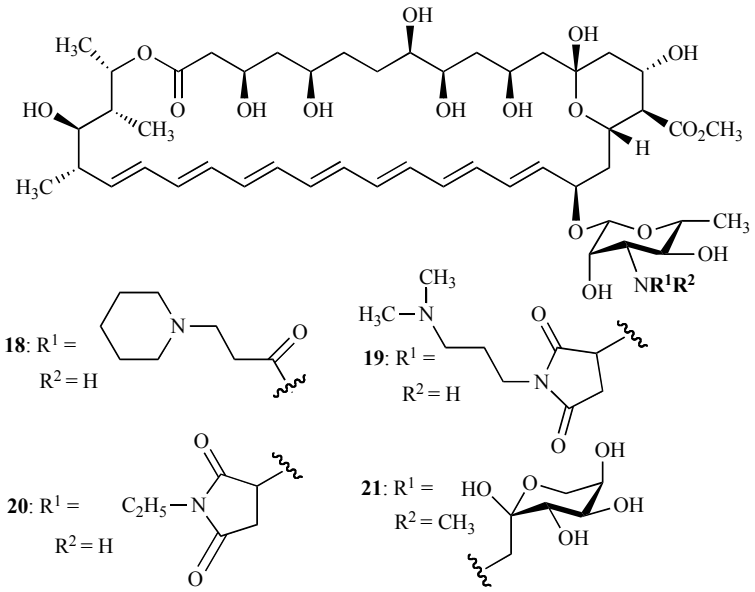


Fig. (3). Structures of derivatives **18-21**.

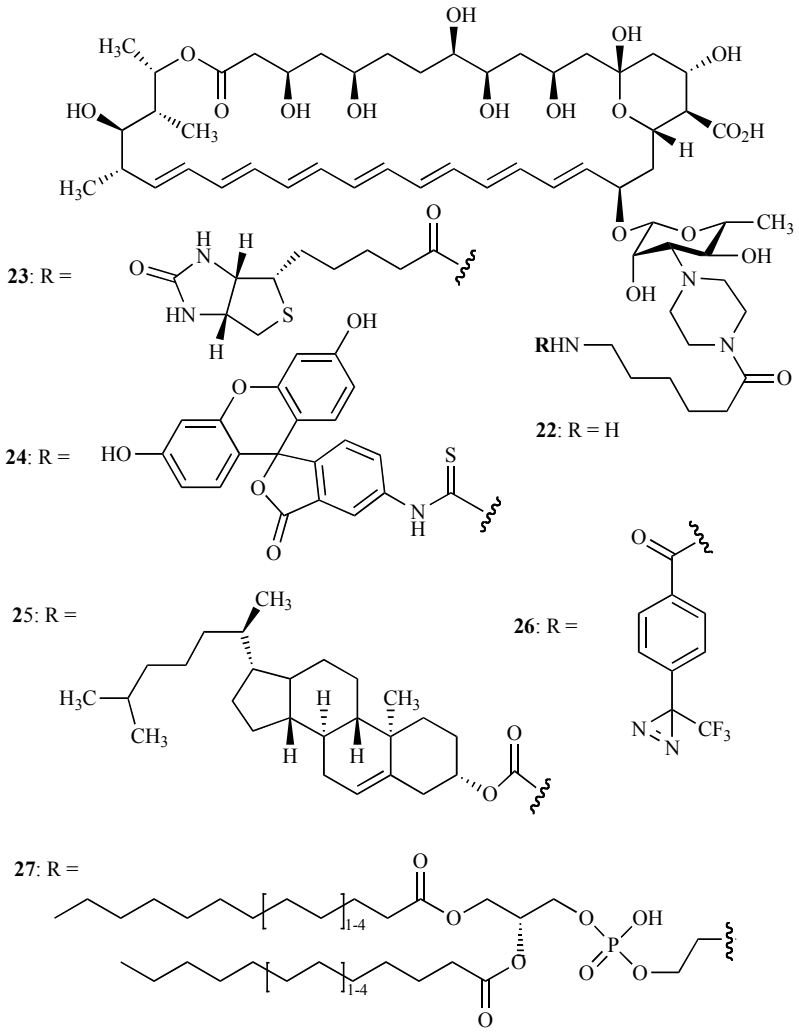


Fig. (4). Structures of derivatives **22**-**27**.

preserves its high antifungal activity (*Candida albicans*: MIC = 6.4 μg/ml; *Candida parapsilosis*: MIC = 1.6 μg/ml; *Candida neoformans*: MIC = 0.8 μg/ml).

3. POLYMERIC CONJUGATES OF AMB

3.1. Conjugates of AMB with Substituted PEGs

Currently used clinical formulations of AMB with polymeric carriers are of non-covalent nature [17-20]. However, over past several years some papers have appeared that describe syntheses and characterization of conjugates in which AMB and PEG are connected by a covalent bond. For medical applications of substituted PEGs the following properties are the most important: they are non-toxic for molecular weights above 400, non-immunogenic and non-antigenic, i.e. resistant to recognition by the immunity system of organism [55-58]. In our introductory paper [59] we described synthesis of a conjugate in which ca 50 mol % AMB is bound by covalent carbamate link to mPEG, and the remaining 50 mol % is linked non-covalently in this conjugate. This non-covalent bond is most probably realized by π–π interactions and/or by hydrogen bonds between the covalently linked and monomeric AMB molecules. This interaction is probably analogous to that [60] encountered in the case of aggregated forms of AMB Fig. (**7**).

Conjugate **31** possesses a very good solubility in water and its spectrum of antimycotic activity is similar to that of

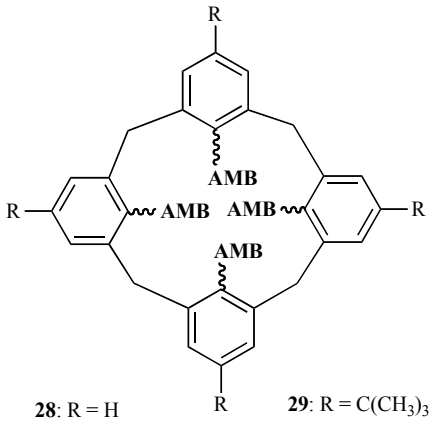
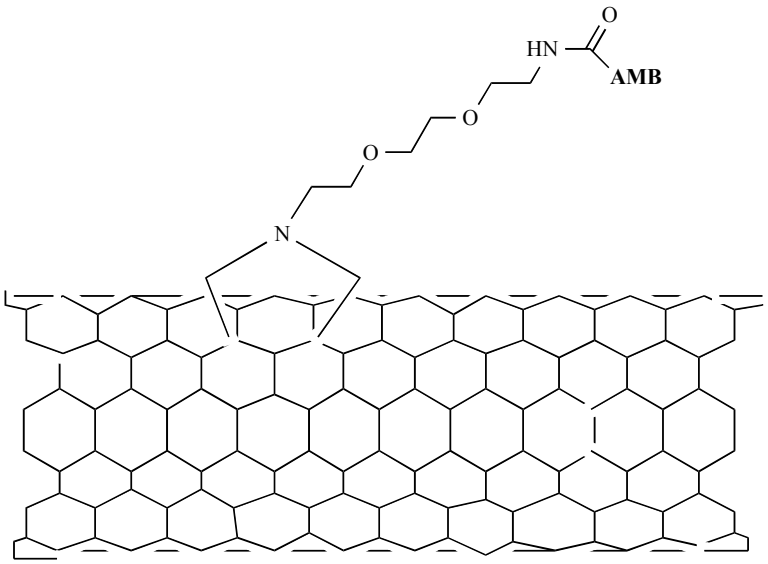


Fig. (5). Structures of conjugates of AMB with calix[4]arenes **28** and **29**.

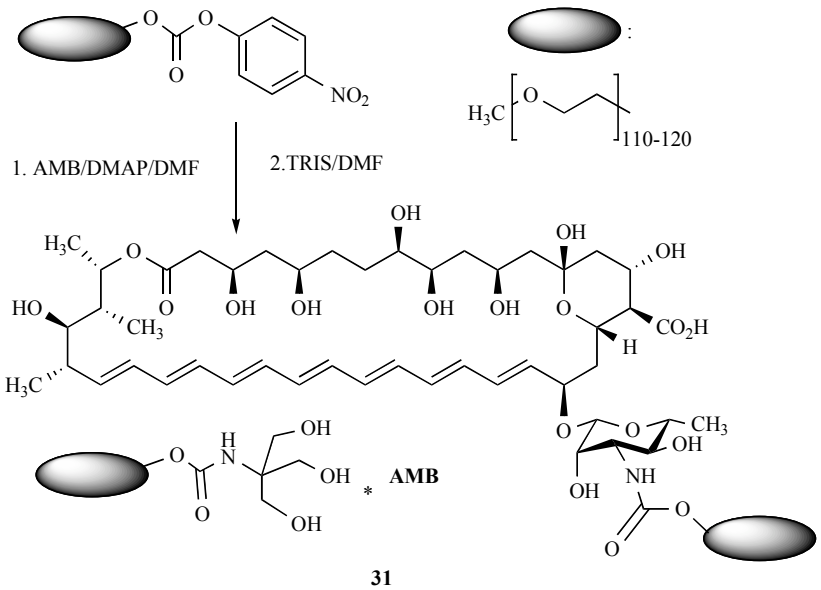


30: R = $C(CH_3)_3$

Fig. (6). AMB covalently linked to carbon nanotube **30**.

the liposomal and deoxycholate formulation. At present, pharmacokinetic studies of this conjugate *in vivo* on animal models are being performed. This work was continued by R. B. Greenwald's team [61, 62]. They prepared a series of conjugates with PEG (e.g. **32**, **33**), where AMB is linked by a labile carbamate bond that is sensitive to blood esterases and decomposes in blood plasma *via* elimination mechanism to give free AMB (**32**: $t_{1/2}$ = 2.9 hours, **33**: $t_{1/2}$ = 4 hours; blood plasma, rat) Fig. (**8**).

This labile bond between PEG and AMB is designed in such a way that the first, slowest step involves hydrolysis of ester linkage by the action of blood esterases. The primary product – prodrug – in the second step undergoes a very rapid base-catalyzed 1,6-benzyl elimination [63] giving quinonemethide and carbamic acid of AMB. The quinonemethide is immediately hydrated and the carbamic acid of AMB is rapidly decomposed into carbon dioxide and free AMB [61, 62]. This effective substance-releasing system can be suitable, e.g., for targeted distribution of cytostatics [64, 65], but it cannot be applied generally to any groups of drugs. For example, in the case of the conjugates **32**-**33** this way of drug release leads to a relatively fast increase of AMB concentration in blood circulation system. The AMB released in this way undergoes distribution [66] among the lipoproteins present in blood (HDL, LDL). Fast increase of the AMB-LDL complex concentration in kidneys results in nephrotoxic action [66, 67]. AMB is considered to have a dual mechanism



31

Fig. (7). Preparation and structure of covalent/noncovalent conjugate **31**.

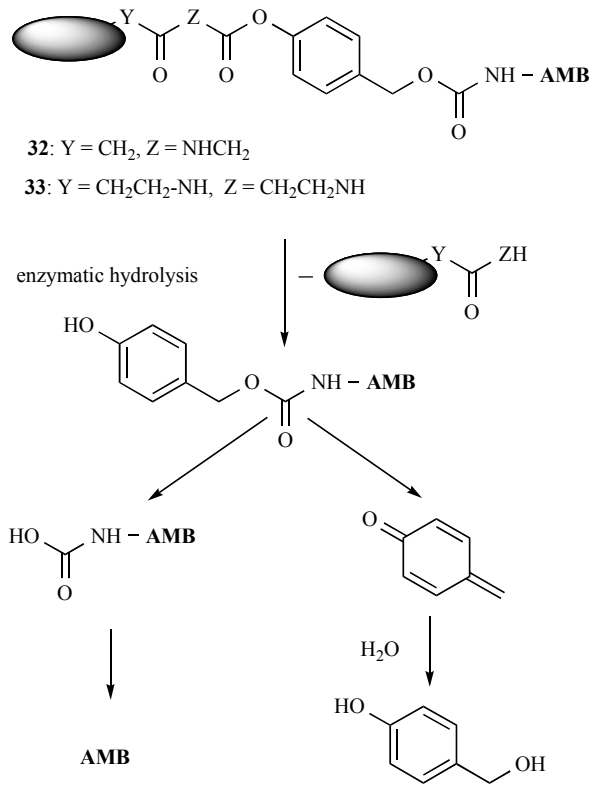


Fig. (8). Structure and principle of release of AMB from conjugates **32** and **33**.

of nephrotoxicity. First, it causes direct damage of the distal tubules presumably due to non-specific interactions with cholesterol (influenced by LDL). Second, it causes constriction of afferent renal vascular perfusion, which in a dehydrated patient can lead to acute renal failure even after a single dose of amphotericin B. Prolonged treatment of AMB can manifest a compensatory mechanism (renal-tubular glomerular feedback) with ongoing tubular damage [10, 11, 13]. This is why many clinicians administer saline before and after AMB infusions to blunt this feedback mechanism [13]. Therefore, targeted conjugates of AMB with PEG were prepared and characterized: in these conjugates AMB is bound to prevent its cleavage in blood circulation system after intravenous application and thus ensuring its targeted release at the site attacked by fungal pathogens [68-70]. The first way to targeted antimycotic effect is based on utilization of the local pH decrease at the site of action of fungal pathogen [70]. Conjugates **34-36** which contain a pH-sensitive imino linkage were prepared for this purpose Fig. (**9**).

Conjugates **34-36** are relatively stable in phosphate buffer at pH = 7.4 (37 °C), i.e. less than 5 mol % of free AMB is cleaved from all these conjugates within 24 hours. Hydrolysis of imino bond takes place in acidic phosphate buffer (pH = 5.5, 37 °C) with formation of free AMB. The rate of acid-catalyzed hydrolysis is controlled by substitution of the benzene ring ($t_{1/2}$ = 2 min for the ester and amide; $t_{1/2}$ = 45 min for the ether) Fig. (**9**). However, the acid-catalyzed hydrolysis does not depend on the molecular mass of the PEGs used. The conjugates having the ester linkage are enzymatically cleaved in human blood plasma and/or blood serum at pH = 7.4 (37 °C), the half-lives 2-5 hours being independent of the molecular mass of PEG (M_w = 5 000, 10 000, 20 000). The hydrolysis of ester linkage primarily gives a relatively stable prodrug, namely 4-carboxybenzylidene-imino-AMB, which is decomposed to AMB and 4-formylbenzoic acid only at pH < 7 ($t_{1/2}$ = 2 min, pH = 5.5, 37 °C). The targeted AMB release takes place only *via* acid-catalyzed hydrolysis of imino linkage either directly from the

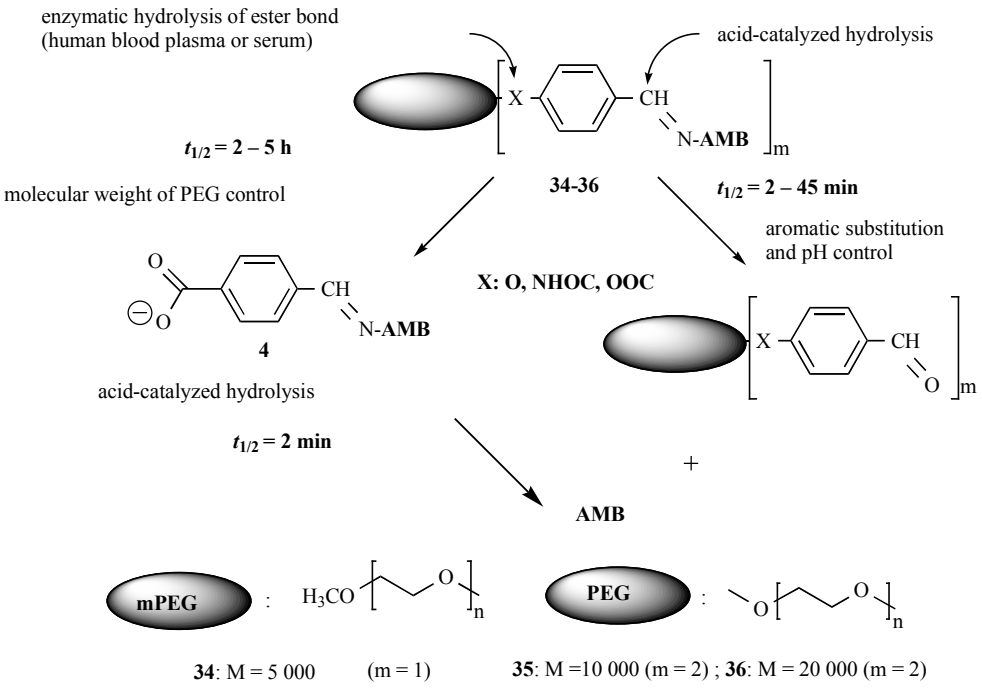
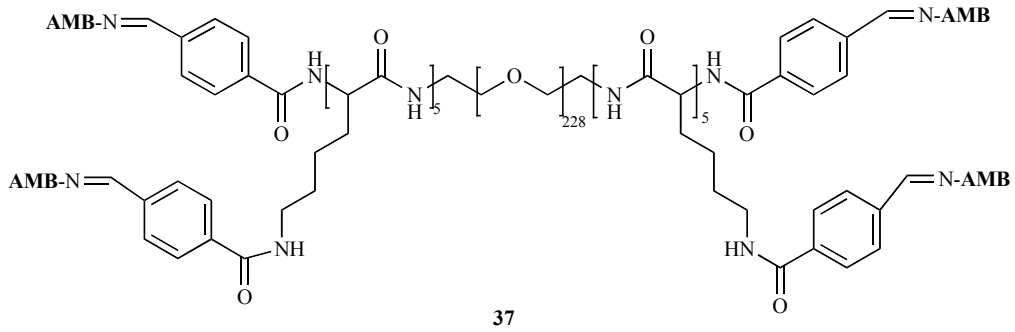


Fig. (9). Structure and principle of AMB release from pH-sensitive conjugates **34-36**.



37

Fig. (10). Structure of conjugate **37** (poly(ethylene glycol)-[*b*-poly(L-lysine)$_5$]$_2$-(AMB)$_{12}$).

polymeric conjugate or from the released prodrug Fig. (**9**). The values of LD_{50} determined *in vivo* (mouse) are 20.7 mg/kg and 40.5 mg/kg for the conjugates with ester linkage (M_w = 10 000 and 5 000), which means that they are ca 6-11 times less toxic than the free AMB [68]. The subsequent work [69] describes preparation and characterization of analogous conjugate **37** (poly(ethylene glycol)-[*b*-poly((L-lysine)$_5$]$_2$-(AMB)$_{12}$; M_w = 26 700) Fig. (**10**). In a phosphate buffer (pH = 5.5, 37 °C), the imine bonds undergo acid-catalyzed hydrolysis with subsequent release of free AMB ($t_{1/2}$ = 2 min.). The LD_{50} value of **37** determined *in vivo* (mouse) is 45 mg/kg [69].

The second synthesized type of the covalent conjugate [70] was **38** (M_w = 25 160). In this conjugate, four AMB molecules are linked with sPEG. Conjugate **38** contains *β*-D-glucopyranoside molecular trigger that is sensitive to *β*-glucosidases (E.C.3.2.1.21). Moreover, the conjugate contains two carbamate linkages: the trigger is bound to the polymer by a stable carbamate linkage, and AMB is bound to phenyl-*β*-D-glucopyranoside fragment by a labile carbamate linkage. The enzymatic hydrolysis of *β*-glucosidic trigger gives glucose, and the subsequent fast 1,6-elimination releases free AMB Fig. (**11**).

The structure design of conjugate **38** Fig. (**11**) was inspired by the fact that many parasitic fungal pathogens, e.g., genera *Aspergillus*, *Candida* or *Trichosporon* possess specific hydrolases *β*-glucosidases (E.C.3.2.1.21) in their enzymatic outfit [71]. In the case of human organism, however, *β*-glucosidases are not present in healthy tissues [72, 73]. It can be presumed that – in contrast to the current intravenous forms used and the first types of covalent conjugates [17-22] – conjugates **37** and **38** should possess targeted release AMB, i.e. at the site of tissue attacked by fungal pathogen. This newly designed system can be applied generally also to

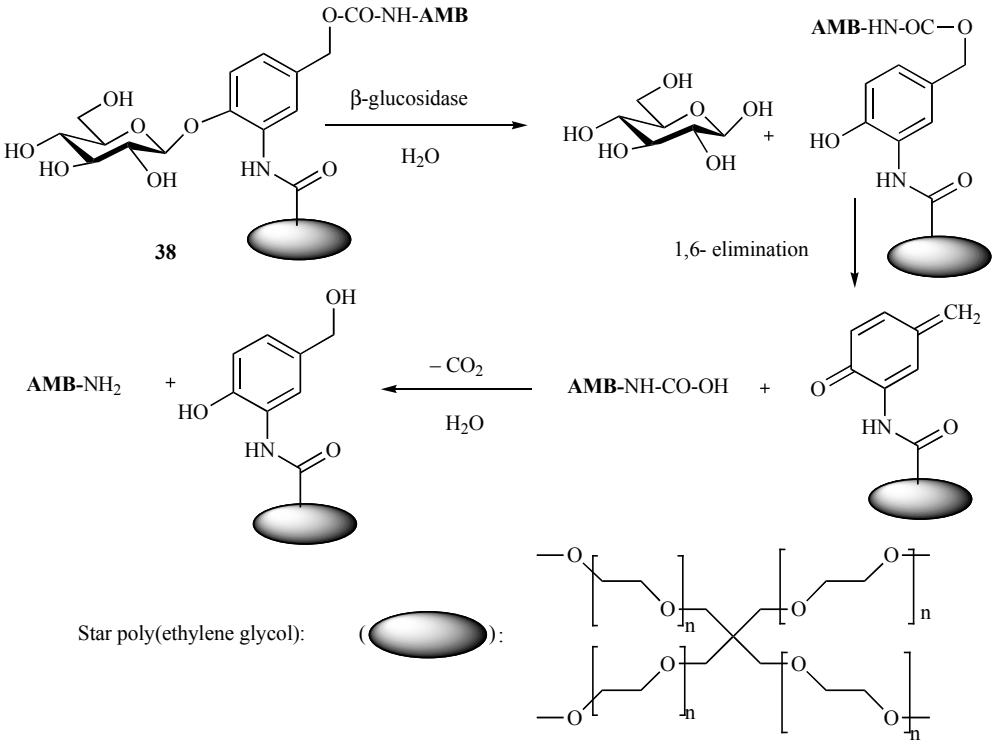


Fig. (11). Structure and principle of targeted AMB release from *β*-glucosidase-sensitive conjugate **38**.

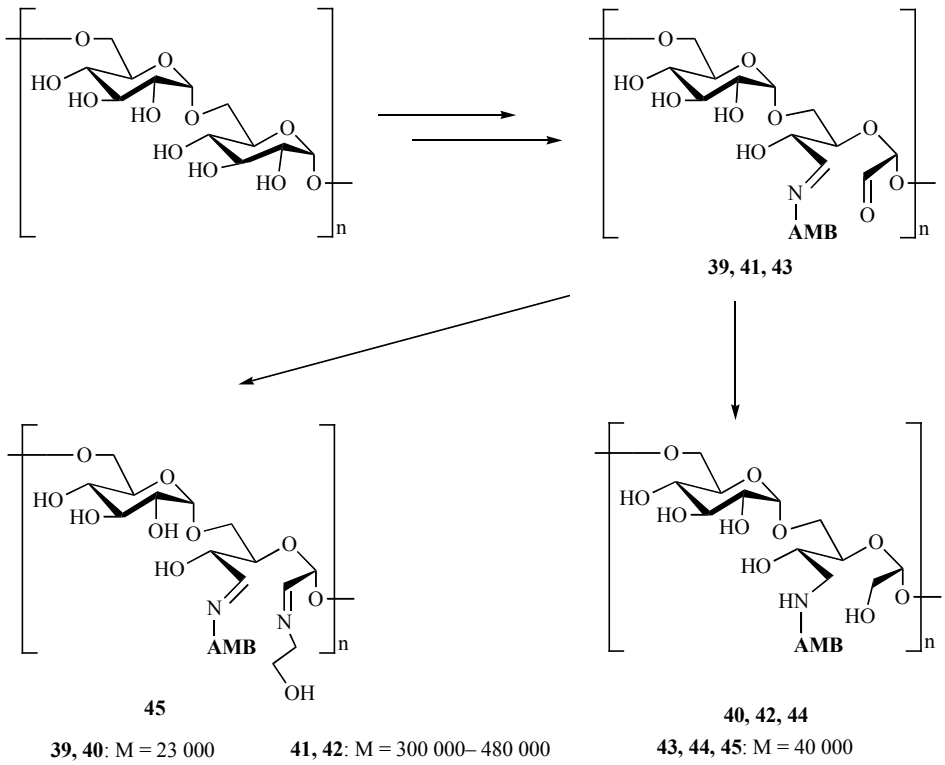


Fig. (12). Structures of conjugates **39-45**.

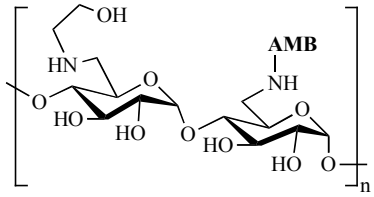
the other drugs with antifungal activity. Of course, suitability of practical application will require further studies on animal models and their critical evaluation.

3.1. Conjugates of AMB with Polysaccharides

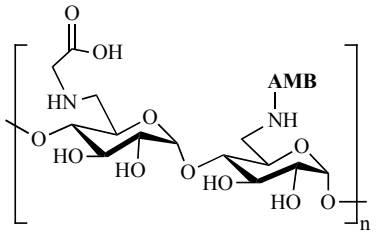
Apart from poly(ethylene glycols), also bio-compatible polysaccharides were used as polymeric carriers for AMB [74-77]. Synthesized conjugate **39** (M_w = 23 000) contains oxidized (KIO_4) arabinogalactan, and AMB is bound by imino linkage Fig. (**12**) [74]. Conjugate **39** is very well soluble in water and exhibits biological activity comparable with that of AMB (MIC = 2 μg/ml *Candida albicans, Cryptococcus neoormans*, MICs, 0.1 to 0.2 μg/ml).

Reduction ($NaBH_4$) of conjugate **39** gave conjugate **40** Fig. (**12**), which is non-hemolytic and much safer than the micellar AMB-deoxycholate. Conjugate **40** was less toxic than conjugate **39** with mice (the maximum tolerated dose for **40:** 50 mg/kg of body weight), and histopathology indicated no damage to the liver or kidneys. This conjugate, similarly to the liposomal formulation (AmBisome), was more effective than AMB-deoxycholate in prolonging survival. It was more effective than both the liposomal and the deoxycholate formulations in eradicating yeast cells from target organs [74].

Another paper [75] describes preparation of conjugates **41** and **42**, which are structurally similar to conjugates **39** and **40** but possess higher molecular masses (M_w = 300 000-480 000). These conjugates exhibit similar biological properties as the foregoing ones. The *in vitro* release of AMB from both imine **41** and amine **42** conjugates was examined in phosphate buffer (pH = 7.4). The authors report that besides the hydrolysis of imino bond of conjugate **41** there also takes place AMB release from amino conjugate **42**. However, no real mechanism corresponds to hydrolysis of the bond polymer–CH_2–NH–AMB giving AMB–NH_2. Most probably, the authors monitored an increase of undefined low-molecular AMB conjugate, which was probably formed by hydrolytic fragmentation of the polymeric carrier. This error cannot be excluded by the technique used: the drug release studies were conducted in a 2-compartment Perspex diffusion cell separated by a dialysis membrane of MWCO 3500. Free released AMB was estimated by UV absorbance at 405 nm. From our works [59, 68-70] it is obvious that the conjugates prepared by a modification of amino group in AMB possess UV/Vis spectra very similar to those of free AMB. For verification, it was necessary to determine free AMB amount by suitable separation technique (HPLC, GPC). The question that remains to be answered concerns the identity of the lower-molecular AMB bioactive conjugate. Probably it was a conjugate with saccharide fragments linked to the amino group of AMB. This conclusion is also confirmed by an earlier-published paper [76] describing AMB conjugates with oxidized dextran (M_w = 40 000). The authors of this work concluded that the imine conjugate **43** released free AMB while the amine conjugate **44** did not. The dextran-AMB imine conjugate **43**, which contains unreacted aldehyde groups, was then modified with ethanolamine to give conjugate **45** Fig. (**12**). This conjugate **45** was compared to dextran-AMB amine **44** and imine **43** conjugates. Modification of an aldehyde groups with ethanolamine reduced the toxicity at least 15-times. The effect on *Leishmania major* parasites was 5 times higher than that of the dextran-AMB amine conjugate **44**. The conjugate **45** was at least 15 times less



46



47

Fig. (13). Structures of conjugates **46-47**.

hemolytic than free AMB. It is concluded that the aldehyde groups may contribute to the cell toxicity. The results have direct implications toward the safety of AMB-polysaccharide conjugates used against fungal and leishmanial infections. Another possible method of linking AMB to arabinogalactan *via* tosylate or mesylate derivatives was described in Ref. [77]. The prepared conjugates **46** and **47** Fig. (**13**) were soluble in water and exhibited improved stability in aqueous solutions as compared to the unbound drug. The conjugates showed comparable inhibition concentration values against the pathogenic yeast *Candida albicans*, and against *Leishmania major* parasites. They were about 60 times less hemolytic against sheep erythrocytes than the free AMB, and less toxic when injected i.v. to BALB/c mice [77].

CONCLUSION

The survey presented shows that the covalent derivatives of AMB prepared by the chemical modification of its functional groups can exhibit both a significant lowering and a distinct increase of their biological activity as compared with free AMB. Apart from the finding that the positively charged nitrogen atom of amino group in AMB is necessary for effective interaction with ergosterol, it is impossible to state any further general principles of AMB modification that would result in increase of biological activity. The set of AMB derivatives synthesized represents promising analogs of AMB with a high potential of biological activity. The same is true also of the covalent AMB conjugates with other molecules, carbon nanotubes and polymers. The large therapeutic potential of these systems can be expected with AMB-carbon nanotubes conjugates or with AMB-β-glucosidase-sensitive conjugates, where the principle of targeted delivery of AMB can be generally applicable also to the other antimycotics. Polymeric conjugates of AMB with modified polysaccharides represent another promising way to increase the therapeutic index of AMB. The imino linkage between AMB and polymeric carrier also represents a potential alternative for targeted release of AMB to tissues with pathologically lowered pH values. Despite all the mentioned advantages of the derivatives and polymeric conjugates of AMB, it has to be stated that the reported scale of these analogs does not reflect their broad clinical applications. However clinical applications require complete tests in accordance with existing legislation. On the other hand, in future these derivatives and conjugates will offer new possibilities of dealing with emerging resistance [78] of fungal pathogens towards AMB alone. We can expect further increase in a number of published works dealing with new delivery systems for antimycotics similarly as in the case of cancerostatic drugs [64, 65]. These papers will be probably oriented to polymeric nanoparticles (liposomes, nanotubes, dendrimers) and many of them will certainly show interesting properties. Research in this field is proceeding at a rapid pace, increasing the possibility that one or more of these vector-cargo complexes could reach the clinic in the not distant future.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the Project of Ministry of Education, Youth and Sports No. MSM 002 162 7501.

ABBREVIATIONS

AMB	=	Amphotericin B
ABLC	=	Amphotericin B lipid complex
BALB/c	=	Albino strain of laboratory mouse
DMAP	=	4-Dimethylaminopyridine
DMF	=	Dimethylformamide
HDL	=	High-density-lipoprotein
LDL	=	Low-density-lipoprotein
MWCO	=	Molecular weight cut-off
PEG	=	Poly(ethylene glycol)
mPEG	=	Methoxy poly(ethylene glycol)
sPEG	=	Star poly(ethylene glycol) [pentaerythritol poly(ethylene glycol)ether]
TRIS	=	2-Amino-2-hydroxymethyl-propane-1,3-diol

REFERRENCES

[1] Trejo, W.; Bennett, R. Streptomyces nodosus sp.n. - amphotericin-producing organism. *J. Bacteriol*., **1963**, *85*, 436-8.

[2] Mechlinski, W.; Schaffner, C. P.; Ganis, P.; Avitabile, G. Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. *Tetrahedron Lett.*, **1970**, *11,* 3873-6.

[3] Woodward, R. B. Structure and biogenesis of the macrolides - a new class of natural substances. *Angew. Chem.*, **1957**, *69*, 50-8.

[4] Ceregheti, D. M.; Carreira, E. M. Amphotericin B: 50 years of chemistry and biochemistry. *Synthesis*, **2006**, 914-42.

[5] Diamond, R. D. Fungal-infections in the immunocompromised host – discussion. *Rev. Infect. Dis*., **1991**, *13*, 504-8.

[6] Lampen, J. O.; Arnow, P. M.; Borowska, Z.; Laskin, A. Location and role of sterol at nystatin-binding sites. *J. Bacteriol*., **1962**, *84*, 1152-8.

[7] Kinsky, S. C. Nystatin binding by protoplasts and a particulate fraction of neurospora crassa, and a basis for selective toxicity of polyene antifungal antibiotics. *Proc. Natl. Acad. Sci. USA*, **1962**, *48*, 1049-56.

[8] Kinsky, S. C.; Luse, S. A.; Van Deen, L. L. M. Interaction of polyene antibiotics with natural and artificial membrane systems. *Fed. Proc.*, **1966**, *25***,** 1503-12.

[9] Hartsel, S. C.; Benz, S. K.; Peterson, R. P.; Whyte, B. S. Potassium-selective amphotericin-B channels are predominant in vesicles regardless of sidedness. *Biochemistry*, **1991**, *30*, 77-82.

[10] Clements, J. S.; Peacock J. E. Amphotericin-B revisited - reassessment of toxicity. *Am. J. Med.,* **1990**, *88*, 22-7.

[11] Wasan, K. M.; Wong, J. C.; Corr, T.; Pritcharrd, S. Role of plasma lipids and lipoproteins in predicting amphotericin B-induced nephrotoxicity in pediatric oncology patients. *Cancer Chemother. Pharmacol.*, **2006**, *57*, 120-4.

[12] Schneider, H.; Brown, J. R.; Muirhead, E. E. Use of Fungizone and polymyxin B in isolation of naturally infected tissues. *Nature*, **1962**, *195*, 396-7.

[13] Eriksson, U.; Seifert, B.; Schaffner, A. Comparison of effects of amphotericin B deoxycholate infused over 4 or 24 hours: randomised controlled trial. *Br. J. Med.,* **2001**, 322, *579-82*.

[14] Vandermeulen, G.; Rouxhet, L.; Arien, A.; Brewster, M. E.; Préat, V. Encapsulation of amphotericin B in poly(ethylene glycol)-block-poly(ε-caprolactone-co-trimethylenecarbonate) polymeric micelles. *Int. J. Pharm.*, **2006**, *309*, 234-40.

[15] Espuelas, M. S.; Legrand, P.; Cheron, M.; Barratt, G.; Puisieux, F.; Renedo, M. J., Devissaguet, J. P., Irache, J. M. Interaction of amphotericin B with polymeric colloids - A spectroscopic study. *Colloids Surf B.,* **1998**, *11*, 141-51.

[16] Espuelas, M. S.; Legrand, P.; Cheron, M.; Barratt, G.; Puisieux, F.; Renedo, M. J., Devissaguet, J. P., Irache, J. M. Interaction of amphotericin B with polymeric colloids: 2. Effect of poloxamer on the adsorption of amphotericin B onto poly(epsilon-caprolactone) nanospheres. *Colloids Surf B.,* **1998**, *11*, 203-12.

[17] Janoff, A. S.; Perkins, W. R., Saleton, S. L.; Swenson, C. E. Amphotericin B lipid complex (ABLC(TM)): A molecular rationale for the attenuation of amphotericin B related toxicities. *J. Liposome Res.*, **1993**, *3*, 451-71.

[18] Rapp, R. P.; Gubbins, P. O.; Evans, M. E. Amphotericin B lipid complex. *Ann. Pharmacother*., **1997**, *31*, 1174-86.

[19] Adler-Moore, P.; Proffitt , R. T. J. Development, characterization, efficacy and mode of action of AmBisome, a unilamellar liposomal formulation of amphotericin B. *Liposome Res.*,**1993**, *3*, 429-50.

[20] Janoff, A. S.; Boni, L. T.; Poescu, M. C.; Minchey, S. R.; Cullis, P. R.; Madden, T. D.; Tarashi, T. F.; Gruner, S. M.; Shyamsunder, E. Unusual lipid structures selectively reduce the toxicity of amphotericin B. *Proc. Natl. Acad. Sci. U. S. A.,***1988**, *85*, 6122-6.

[21] Moribe, K.; Mararuyama, K.; Iwatsuru M. Molecular localization and state of amphotericin B in PEG liposomes. *Int. J. Pharm.,* **1999**, *193*, 97-106.

[22] Carrillo-Muñoz, X. A. J.; Quindós, G.; Tur, C.; Ruesga, M. T.; Miranda, Y.; del Valle, O.; Cossum, P.A.; Wallace, T. L. In-vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole. *J. Antimicrob. Chemother*., **1999,** *44*, 397-401.

[23] Fisher, P. B.; Goldstein, N. I.; Bryson, V.; Schaffner C. P. Reduced toxicity of amphotericin B methyl ester (AME) *vs*. amphotericin B and fungizone in tissue culture. *In Vitro,* **1976**, *12*, 133-40.

[24] Marmer, D. J.; Fields, B. T. Jr.; France, G. L.; Steele, R. W. Ketoconazole, amphotericin B, and amphotericin B methyl ester: Comparative *in vitro* and *in vivo* toxicological effects on neutrophil function. *Antimicrob. Agents Chemother*., **1981**, *20*, 660-5.

[25] Waheed, A. A.; Ablan, S. D.; Mankowski, M. K.; Cummins, J. E.; Ptak, R. G.; Schaffner, C. P.; Freed, E. O. Inhibition of HIV-1 replication by amphotericin B methyl ester: Selection for resistant variants. *J. Biol. Chem.,* **2006**, 28699-711.

[26] Li, J.; Zhu, H.-Q.; Li, J.-Y.; Jin, S.-H.; Hu, C.-Q. Isolation, structure elucidation and activity of an unknown impurity of amphotericin B. *J. Antibiot.*, **2007***, 60,* 272-6.

[27] Belakhov, V.V.; Shenin, Yu. D. Synthesis and antifungal activity of N-benzyl derivatives of amphotericin B. *Pharm. Chem. J.*, **2007**, *41,* 362-6.

[28] Czerwiński, A.; Grzybowska, J.; Borowski, E. *J.* N-dimethylaminoacyl derivatives of polyene macrolide antibiotics. *Antibiot.*, **1986**, *39*, 1025-7.

[29] Chéron, M.; Cybulska, B.; Mazerski, J.; Grzybowska, J.; Czerwiński, A.; Borowski, E. Quantitative structure-activity relationships in amphotericin B derivatives. *Biochem. Pharmacol.*, **1988**, *37*, 827-36.

[30] Hac-Wydro, K.; Dynarowicz-Latka, P.; Grzybowska, J.; Borowski, E. How does the *N*-acylation and esterification of amphotericin B molecule affect its interactions with cellular membrane components - The Langmuir monolayer study. *Colloids Surf., B,* **2005**, *46*, 7-19.

[31] Kaouass, W.; Gamache, I.; Ramotar, D.; Audette, M.; Poulin, R. The spermidine transport system is regulated by ligand inactivation, endocytosis, and by the Npr1p Ser/Thr protein kinase in Saccharomyces cerevisiae. *J. Biol. Chem.,* **1997**, *273*, 2109.

[32] Paquet, V.; Carreira, E. M. Significant improvement of antifungal activity of polyene macrolides by bisalkylation of the mycosamine. *Org. Lett.,* **2006**, *8*, 1807-17.

[33] Paquet, V.; Volmer, A. A.; Carreira, E. M. Synthesis and *in vitro* biological properties of novel cationic derivatives of amphotericin B. *Chem. Eur. J.,* **2008**, *14*, 2465-81.

[34] Graybill, J. R.; Najvar, L. K.; Fothergill, A; Hardin, T.; Rinaldi, M.; Lanbros, C., Regen S. L. KY-62, a polyene analog of amphotericin B, for treatment of murine candidiasis. *Antimicrob. Agents Chemother.,* **1998**, *42*, 147-50.

[35] Yamashita, K.; Janout, V.; Bernard, E.; Armstrong, D.; Regen, S. L. Micelle/monomer control over the membrane-disrupting properties of an amphiphilic antibiotic. *J. Am. Chem. Soc.,* **1995**, *117*, 6249-53.

[36] Al-Abdely, H. M.; Graybill, J. R.; Bocanegra, R.; Najvar, L.; Montalblo, E.; Regen S. L.; Melby, P. C. Efficacies of KY62 against Leishmania amazonensis and Leishmania donovani in experimental murine cutaneous leishmaniasis and visceral leishmaniasis *Antimicrob. Agents Chemother.,* **1998**, *42*, 2542-8.

[37] Driver, M. J.; Greenlees, A. R.; MacPherson, D. T. A versatile strategy for the derivatisation of the carboxy group of amphotericin B: The preparation of C-16 ketone analogues. *J. Chem. Soc. Perkin Trans.1,* **1992**, 3155-7.

[38] Hunter, P.; Carter, E.; Murdock, L.; Williams, L.; Scott-Wood, G. Ketone analogues of amphotericin B. *Abstract of Papers of 32nd Intersci. Conf. Antimicrob. Agents Chemother.,* **1992**, 104-5.

[39] Costello, B. J.; Driver, M. J., Maclachlan, W. S., Taylor, A. Stereoselective syntheses of the 14-hydroxy epimers of amphotericin B methyl ester. *J. Chem. Soc. Perkin Trans.1,* **1993**, 1829-30.

[40] Corbett, D.F.; Dean, D. K.; Greenless, A. R.; Macpherson, D. T. Synthesis and antifungal activity of C-16 oximino and vinyl amphotericin B derivatives. *J. Antibiot*., **1995**, *48*, 509-15.

[41] Czub, J.; Borowski, M.; Baginski, M. Interactions of amphotericin B derivatives with lipid membranes-A molecular dynamics study. *Biochim. Biophys. Acta*, **2007**, *1768*, 2616-26.

[42] Szlinder-Richert, J.; Cybulska, B.; Grzybowska, J.; Bolard, J.; Borowski, E. Interaction of amphotericin B and its low toxic derivative, N-methyl-N-D-fructosyl amphotericin B methyl ester, with fungal, mammalian and bacterial cells measured by the energy transfer method. *Farmaco,* **2004**, *59*, 289-96.

[43] Zumbuehl, A.; Stano, P.; Sohrmann, M.; Peter, M.; Walde, P.; Carreira, E. M. A novel strategy for bioconjugation: Synthesis and preliminary evaluation with amphotericin B *Org. Biomol. Chem.,* **2007**, *5,* 1339-42.

[44] Matsuoka, S.; Matsumori, N.; Murata, M. Amphotericin B-phospholipid covalent conjugates: Dependence of membrane-permeabilizing activity on acyl-chain length *Org. Biomol. Chem.,* **2003**, *1,* 3882-4.

[45] Kasai, Y.; Matsumori, N.; Umegawa, Y.; Matsuoka, S.; Ueno, H.; Ikeuchi, H.; Oishi, T.; Murata, M. Self-assembled amphotericin B is probably surrounded by ergosterol: Bimolecular interactions as evidenced by solid-state NMR and CD spectra. *Chem. Eur.J.,* **2008**, *14,* 1178-85.

[46] Umegawa, Y.; Matsumori, N.; Oishi, T.; Murata, M. Amphotericin B covalent dimers with carbonyl-amino linkage: a new probe for investigating ion channel assemblies *Tetrahedron Lett,*. **2007**, *48,* 3393-6.

[47] Tsuchikawa, H.; Matsuhita, N.; Matsumori, N.; Murata, M.; Oishi, T. Synthesis of 28-^{19}F-amphotericin B methyl ester. *Tetrahedron Lett.,* **2006**, *47,* 6187-91.

[48] Rogers, B. N.; Selsted, M. E.; Rychnovsky, S. D. Synthesis and biological evaluation of non-polyene analogs of amphotericin. *Bioorg. Med.Chem. Lett.* **1997**, *7*, 3177-82.

[49] Szpilman, A. M.; Cereghetti, D. M.; Wurtz, N. R.; Manthohorpe, J. M.; Carreira, E. M. Synthesis of 35-deoxy amphotericin B methyl ester: A strategy for molecular editing. *Angew. Chem. Int. Ed.,* **2008**, *47,* 4335-8.

[50] Szpilman, A. M.; Manthohorpe, J. M.; Carreira, E. M. Synthesis and biological studies of 35-deoxy amphotericin B methyl ester. *Angew. Chem. Int. Ed.,* **2008**, *47,* 4339-42.

[51] Paquet, V.; Zumbuehl, A.; Careira, E. M. Biologically active amphotericin B-calix[4]arene conjugates. *Bioconjug. Chem.,* **2006**, *17*, 1460-3.

[52] Wu, W.; Wieckowski, S.; Pastorin, G.; Benincasa, M.; Klumpp, C.; Briand, J.; Gennaro, R.; Prato, M.; Bianco, A. Targeted delivery of amphotericin B to cells by using functionalized carbon nanotubes. *Angew. Chem., Int. Ed.,* **2005**, *44,* 6358-62.

[53] Kostarelos, K.; Lacerda, L.; Pastorin, G.; Wu, W.; Wieckowski, S.; Luangsivilay, J.;Godefroy, S.; Pantarotto, D.; Briand, J.-P.; Muller, S.; Prato, M.; Bianco, A. Cellular uptake of functionalized carbon nanotubes is independent of functional group and cell type. *Nat. Nanotechnol.,* **2007**, *2*, 108-13.

[54] Prato, M.; Kostarelos, K.; Bianco, A. Functionalized carbon nanotubes in drug design and discovery. *Acc. Chem. Res.,* **2008,** *41*, 60-8.

[55] Zalipsky, S. Functionalized poly(ethylene glycol) for preparation of biologically relevant conjugates. *Bioconjug. Chem.,* **1995**, 150-65.

[56] Harris, J. M. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Application*; Harris, J. M., Ed.; Plenum Press: New York, **1992**.

[57] Cölfen, H. Double-hydrophilic block copolymers: Synthesis and application as novel surfactants and crystal growth modifiers. *Macromol. Rapid Commun*., **2001**, *22*, 219-52.

[58] Sedlák, M. Recent advances in chemistry and applications of substituted poly(ethylene glycol)s. *Collect. Czech. Chem. Commun*., **2005**, *70*, 269-91.

[59] Sedlák, M.; Buchta, V., Kubicová, L.; Šimůnek, P.; Holčapek, M.; Kašparová, P. Synthesis and characterisation of a new amphotericin B-methoxypoly(ethylene glycol) conjugate. *Bioorg. Med. Chem. Lett.,* **2001**, *11*, 2833-5.

[60] Gaboriau, F.; Chéron, M.; Leroy, L.; Bolard, J. Physico-chemical properties of the heat-induced 'superaggregates' of amphotericin B. *Biophys. Chem.,* **1997**, *66*, 1-12.

[61] Conover, C. D.; Zhao, H.; Clifford, B.; Longley, C. B.; Shum, K. L.; Greenwald. R. B. Utility of poly(ethylene glycol) conjugation to create prodrugs of amphotericin B. *Bioconjug. Chem.,* **2003**, *14*, 661-6.

[62] Greenwald, R. B.; Choe, Y. H.; McGurie, J.; Conover, C. D. Effective drug delivery by PEGylated drug conjugates. *Adv. Drug Deliv. Rev.,* **2003**, *55*, 217.-50.

[63] Wakselman, M. 1,4-Eliminations and 1,6-eliminations from hydroxy-substituted and amino-substituted benzyl systems - chemical and biochemical applications. *Nouv. J. Chim.,* **1983**, *7*, 439-47.

[64] Ulbrich, K.; Šubr, V. Polymeric anticancer drugs with pH-controlled activation. *Adv. Drug Deliv. Rev*., **2004**, *56*, 1023-50.

[65] Pechar, M.; Braunová, A.; Ulbrich, K.; Jelínková, M.; Říhová, B. Poly(ethylene glycol) - Doxorubicin conjugates with pH-controlled activation. *J. Bioact. Compat. Polym*., **2005**, *20*, 319-41.

[66] Sivak, O.; Lau B.; Patankar, N.; Wasan, K. M. Unidirectional inhibition of lipid transfer protein I-mediated transfer of cholesteryl esters between high-density and low-density lipoproteins by amphotericin B lipid complex. *Pharm. Res.,* **2004**, *21*, 2336-9.

[67] Wasan, K. M.; Wong, J. C.; Corr, T.; Pritcharrd, S. Role of phospholipid transfer protein on the plasma distribution of amphotericin B following the incubation of different amphotericin B formulations. *Cancer Chemother. Pharmacol.,* **2006**, *57*, 120.

[68] Sedlák, M.; Pravda, M.; Staud, F.; Kubicová, L.; Týčová, K.; Ventura, K. Synthesis of pH-sensitive amphotericin B-poly(ethylene glycol) conjugates and study of their controlled release *in vitro*. *Bioorg. Med. Chem.,* **2007**, *15*, 4069-76.

[69] Sedlák, M.; Pravda, M.; Kubicová, L.; Mikulčíková, P.; Ventura, K. Synthesis and characterisation of a new pH-sensitive amphotericin B-poly(ethylene glycol)-b-poly(l-lysine) conjugate. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 2554-7.

[70] Sedlák, M.; Drabina, P.; Bílková, E.; Šimůnek, P.; Buchta,V. New targeting system for antimycotic drugs: β-glucosidase sensitive amphotericin B-star poly(ethylene glycol) conjugate. *Bioorg. Med. Chem. Lett.,* **2008**, *18*, 2952-6.

[71] Li, Y-K.; Chir, J.; Chen, F-Y. Catalytic mechanism of a family 3 β-glucosidase and mutagenesis study on residue Asp-247. *Biochem. J*., **2001**, *335*, 835-40.

[72] Hopfer, U. In *Textbook of Biochemistry with Clinical Correlations*; Devlin, T. M., Ed.; Wiley-Liss: New York, **1992**; pp. 1078-80.

[73] Hamill, J. R. In *Clinical Mycology*; Dismukes, W. E.; Pappas, P. G.; Sobel, J. D. Eds.; Oxford University Press, New York, **2003**, Vol. *4*, pp. 49-58.

[74] Falk, R.; Domb, A. J.; Polacheck, I. A novel injectable water-soluble amphotericin B-arabinogalactan conjugate. *Antimicrob. Agents Chemother.* **1999**, *43*, 1975-81.

[75] Nishi, K. K.; Antony, M.; Mohanan, P. V.; Anilkumar, T. V.; Loiseau, P. M.; Jayakrishman, A. Amphotericin B-gum arabic conjugates: Synthesis, toxicity, bioavailability, and activities against Leishmania and Fungi. *Pharm. Res.,* **2007***, 24*, 971-7.

[76] Sokolsky-Papkov, M.; Domb, A. J.; Golenser, J. Impact of aldehyde content on amphotericin B - Dextran imine conjugate toxicity. *Biomacromol.,* **2006**, *7,* 1529-35.

[77] Ehrenfreund-Kleinman, T.; Golenser, J.; Domb, A. J. Conjugation of amino-containing drugs to polysaccharides by tosylation: Amphotericin B-arabinogalactan conjugates *Biomaterials*, **2004**, *25*, 3049-57.

[78] Vanden Bossche, H.; Dromer, F.; Improvisi, I.; Lozano-Chin, M.; Rex, J. H.; Sanglard, D. Antifungal drug resistance in pathogenic fungi. *Med. Mycol.,* **1998**, *36*(Suppl), 119-128.

Received: 08 June, 2009 **Revised: 28 August, 2009** **Accepted: 28 August, 2009**