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Synthesis of fenoprofen and gemfibrozil styrene-maleic acid copolymer conjugates

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Two types of polymer-drug conjugates were synthesized starting from styrene-maleic acid anhydride copolymer (SMA). Fenoprofen and gemfibrozil were chosen as model drugs because of their short plasma half lives. Both drugs were first converted to their 2-aminoethylamides, which possess free amino groups capable of reacting with SMA anhydride rings. By modifying the degree and type of substitution, lipophilic and hydrophilic conjugates were obtained. Drug loading in the conjugates was between 17 and 47%.

Keywords: fenoprofen, gemfibrozil, polymer-drug conjugate, macromolecular prodrug, styrene-maleic acid anhydride copolymer

The efficacy of therapeutics is often diminished by their insolubility, instability, low bioavailability, non-specificity and systemic toxicity. A considerable number of strategies have been developed in order to obtain drug delivery systems for an effective therapy. Attachment of drugs to suitable polymers significantly alters their properties and pharmacokinetics. Polymer conjugation, largely investigated with both protein and low molecular mass drugs, represents a promising method to improve the physicochemical and biopharmaceutical properties of drugs (1, 2). Polymer conjugation can endow derivatives with increased water-solubility and chemical stability, improved pharmacokinetics and distribution profile, reduced side effects, and sometimes properties targeted at the disease site either by active or passive mechanisms (3).

Styrene-maleic acid anhydride copolymer (SMA) belongs to a group of vinylic polymers. It has no teratogenic, and no acute or chronic toxic effects (4, 5). It is currently in clinical trials as an efficient, reversible male contraceptive (6, 7). SMA has also been used for the preparation of chemically and diffusionally controlled polymeric prodrugs of pyrazolone and phenothiazine derivatives (8), barbituric acid and xantine derivatives (9), dopamine (10), ampicillin (11) or acriflavine (12). There are also examples of attachment of some antimicrobial agents such as 4-aminobenzoic acid, 4-hydroxybenzoic acid (9, 13) and 4-aminophenol (14) to SMA. However, the most successful example is attachment of

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the protein drug neocarzinostatin to SMA *via* two amide bonds. Clinical results are extremely promising with a success rate of 70–90% of the treated tumour patients (15–17).

Fenoprofen is a non-steroidal anti-inflammatory drug with analgesic activity (18). It is indicated in musculoskeletal and joint disorders such as osteoarthritis, rheumatoid arthritis and ankylosing spondylitis, as well as in management of mild to moderate pain, fever and inflammation. Gemfibrozil is a lipid-lowering agent, derivative of fibric acid. It is widely used in the treatment of dyslipidaemias and atherosclerosis, especially in lipoprotein disorders characterized by elevation of very-low-density lipoproteins (VLDL) and plasma triglycerides. Due to their relatively short plasma half-life, both drugs should be given in repeated doses to maintain a therapeutic effect. To overcome these problems, a number of prodrugs and structural analogues of fenoprofen and gemfibrozil have been prepared and evaluated. In our previous papers, binding of fenoprofen and gemfibrozil to biodegradable polyaspartamide-type polymers by ester or amide bonds was reported (19–24). Hydrolysis studies showed that drugs could be released from the macromolecular prodrugs after chemical hydrolysis in a wide pH range.

In this paper, we report the reaction of fenoprofen and gemfibrozil amide derivatives with styrene-maleic acid anhydride copolymer and characterization of the resulting polymer-drug conjugates.

EXPERIMENTAL

IR spectra were recorded on a FT-IR Paragon 500 spectrometer (Perkin-Elmer, UK) and UV spectra on a Hewlett Packard 8452A diode array spectrophotometer (Hewlett Packard, Germany). For thin layer chromatography, silica gel sheets Kieselgel 60 F_{254} (Merck, Germany) were used. Solvent systems were cyclohexane/ethyl acetate/methanol (1:3:1), butanol/water/acetic acid (8:1:1) and cyclohexane/ethyl acetate (1:1). UV light (254 nm) and iodine vapour were used for spot detection. Fenoprofen was purchased from Eli Lilly Company (USA). Gemfibrozil was obtained from Lek (Slovenia). The amines were distilled and dried prior to use. All solvents were of analytical grade and dry.

N-*1-benzotriazolecarboxylic acid chloride (1)*

Compound **1** was prepared according to the previously published procedure (25).

*Benzotriazolides of fenoprofen and gemfibrozil (**2a,b**)*

Compounds **2a** and **2b** were prepared by reaction of *N*-1-benzotriazolecarboxylic acid chloride (**1**) and fenoprofen or gemfibrozil, following the published procedures (19, 26).

*2-Aminoethyl fenoprofenamide (**3a**) and 2-aminoethyl gemfibrozilamide (**3b**)*

Compounds **3a** and **3b** were prepared according to the previously published procedures (20, 21).

*Styrene-maleic acid anhydride copolymer (SMA, **4**)*

SMA was prepared following the procedure described by Kovač-Filipović *et al.* (27).

*(Styrene-maleic acid copolymer)-ethylenediamine-fenoprofen conjugate (SMAC-EDA-Fen, **5a**)*

A solution containing 0.220 g (0.0011 mol calculated as a monomer unit) SMA (**4**), 0.310 g (0.0011 mol) **3a** and 0.111 g (0.0011 mol) triethylamine (TEA) in 12 mL *N*,*N*-dimethylformamide (DMF) was stirred at 50 °C for 7 h. After cooling, the solution was poured into 50 mL of hydrochloric acid (w = 1%). Resulting precipitate was filtered off and washed with water until neutral [IR (KBr): ν_{max} 3318, 2932, 1724, 1653, 1582, 1534, 1490, 1244, 1208, 932, 761, 700 cm^{-1}; nitrogen analysis (%) for monomer unit $C_{29}H_{30}N_2O_5$ (486.61): calcd. N 5.76, found: N 5.42; drug loading: 46.9%].

*(Styrene-maleic acid copolymer)-ethylenediamine-gemfibrozil conjugate (SMAC-EDA-Gem, **5b**)*

A solution containing 0.220 g (0.0011 mol calculated as a monomer unit) SMA (**4**), 0.320 g (0.0011 mol) **3b** and 0.111 g (0.0011 mol) TEA in 7 mL DMF was stirred at 50 °C for 7 h. After cooling the solution was poured into 50 mL of hydrochloric acid (w = 1%). Resulting precipitate was filtered off and washed with water until neutral [IR (KBr): ν_{max} 3340, 2951, 1724, 1642, 1613, 1531, 1510, 1454, 1263, 1129, 1040, 702 cm^{-1}; nitrogen analysis (%) for monomer unit $C_{29}H_{38}N_2O_5$ (494.69): calcd. N 5.66, found: N 4.62; drug loading: 41.3%].

*[(Styrene-2-hydroxyethyl maleamide)-(styrene-maleic acid) copolymer]-ethylenediamine-fenoprofen conjugate (SHM-EDA-Fen, **6a**)*

A solution composed of 0.202 g (0.001 mol calculated as a monomer unit) SMA (**4**), 0.093 g (0.00033 mol) **3a** and 0.101 g (0.001 mol) TEA in 7 mL DMF was stirred at 50 °C for 5 h. To the reaction mixture, 1 mL (1.012 g, 0.017 mol) of ethanolamine was added, and the reaction continued for an additional 5 h. After cooling, the solution was poured into 50 mL of hydrochloric acid (w = 1%). Resulting precipitate was filtered off, triturated and washed with water until neutral [IR (KBr): ν_{max} 3412, 2937, 1734, 1654, 1541, 1456, 1243, 702 cm^{-1}; UV: λ_{max} 276 nm (A = 0.497; γ = 240.4 μg mL^{-1}, methanol); drug loading: 29.6%].

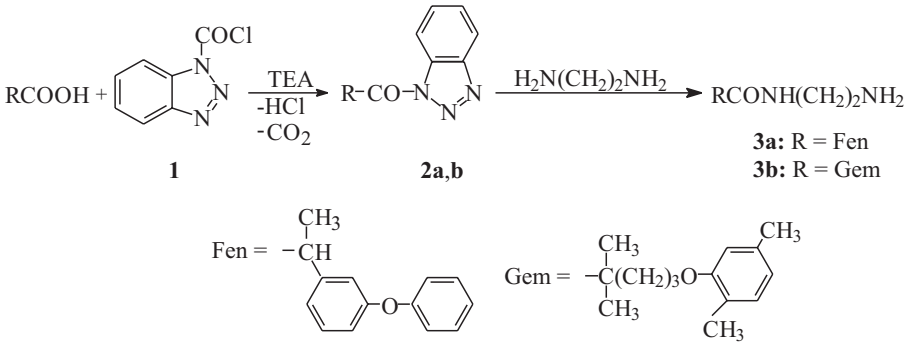
*[(Styrene-2-hydroxyethyl maleamide)-(styrene-maleic acid) copolymer]-ethylenediamine-gemfibrozil conjugate (SHM-EDA-Gem, **6b**)*

A solution made up of 0.202 g (0.001 mol calculated as a monomer unit) SMA (**4**), 0.096 g (0.00033 mol) **3b** and 0.101 g (0.001 mol) TEA in 7 mL DMF was stirred at 50 °C for 3.5 h. To the reaction mixture, 1 mL (1.012 g, 0.017 mol) of ethanolamine was added and the reaction continued for an additional 1 h. After cooling, the solution was poured into 50 mL of hydrochloric acid (w = 1%). Resulting precipitate was filtered off, tritu-

rated and washed with water until neutral [IR (KBr): ν_{max} 3422, 2948, 1730, 1649, 1537, 1454, 1261, 703 cm^{-1}; UV: λ_{max} 276 nm (A = 0.591; γ = 392.0 μg mL^{-1}, H_2O); drug loading: 17.0%].

RESULTS AND DISCUSSION

In the first step, fenoprofen and gemfibrozil reacted with *N*-1-benzotriazolecarboxylic acid chloride (**1**), affording the corresponding benzotriazolides **2a** and **2b**, respectively. The reactions were performed in dry toluene, with the reactants ratio 1:1, in the presence of TEA as HCl acceptor. In the reaction with ethylenediamine, benzotriazolides **2** were transformed into 2-aminoethyl fenoprofenamide (**3a**) and 2-aminoethyl gemfibrozilamide (**3b**), drug derivatives with free amino groups (Scheme 1) (20, 21). In this reaction, amine excess was essential in order to prevent the formation of bis-ethylenediamides.

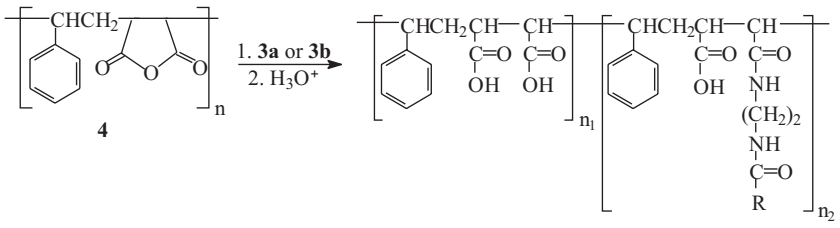


Scheme 1

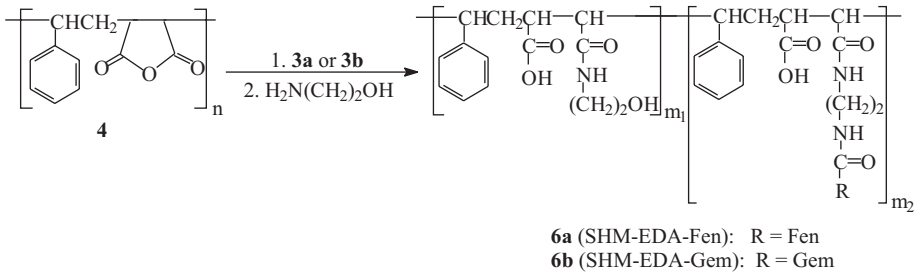
Compounds **3a,b** proved to be useful in the synthesis of polymer-drug conjugates. Free amino groups enabled their reaction with anhydride rings of styrene-maleic acid anhydride copolymer, which was chosen as a polymer component (Schemes 2 and 3). In this way, conjugates SMAC-EDA-Fen (**5a**) and SMAC-EDA-Gem (**5b**) were prepared (Scheme 2). The reactions were performed in dry DMF, at 50 °C, during 7 h. Unreacted anhydride rings were hydrolyzed by hydrochloric acid.

More hydrophilic conjugates bearing free hydroxyethyl groups, SHM-EDA-Fen (**6a**) and SHM-EDA-Gem (**6b**), were prepared by partial aminolysis of SMA with aminoamides **3a** or **3b**, followed by aminolysis of the remaining anhydride units with ethanolamine (Scheme 3). Reactions of SMA with compounds **3** were performed in dry DMF, at 50 °C. The following reactions with ethanolamine proceeded at the same temperature, in excess of the amine in order to prevent cross-linking.

Crude conjugates **5** and **6** were purified by precipitation: their DMF solutions were poured into hydrochloric acid (w = 1%), the precipitated products were filtered off and washed with water. The absence of nonconjugated drug was confirmed by TLC using



Scheme 2



Scheme 3

cyclohexane/ethyl acetate/methanol (1:3:1), butanol/water/acetic acid (8:1:1) and cyclohexane/ethyl acetate (1:1) solvent systems in which the polymer remained at the start and drugs, benzotriazolides **2a**,**b** or aminoamides **3a**,**b**, moved with the mobile phase. IR spectroscopy of conjugates **5a**,**b** and **6a**,**b** showed the absence of cyclic anhydride carbonyl absorption at 1789 and 1855 cm^{-1} and the presence of strong carboxylic acid carbonyl absorption between 1724 and 1734 cm^{-1}. In this way, absence of unreacted anhydride rings was confirmed. IR spectra of the conjugates showed also strong amide carbonyl absorptions at ca 1650 (amide I) and 1540 (amide II), as well as N-H bond absorption at 3330 cm^{-1} (Fig. 1). In addition, conjugates **6a** and **6b** absorbed UV-light in the same absorption ranges as fenoprofen and gemfibrozil, whereas SMA showed no absorption at these wavelengths. These IR and UV data could be considered as an additional proof that drugs were covalently bound to the polymer in the prepared conjugates.

Drug loadings in conjugates **5a** and **5b** were calculated from the nitrogen content and were found to be 46.9 and 41.3%, respectively. The amount of drug in conjugates **6a** and **6b** was estimated by UV-spectroscopy using the molar absorption coefficient of fenoprofen in H_2O (ε_{272} = 1748 $dm^3\ mol^{-1}\ cm^{-1}$) and gemfibrozil in 96% EtOH (ε_{276} = 1866 $dm^3\ mol^{-1}\ cm^{-1}$). Drug loadings of fenoprofen and gemfibrozil in conjugates **6a** and **6b** were 29.6 and 17.0%, respectively.

The prepared polymer-drug conjugates differed in solubility. Conjugates **5a,b** were poorly soluble in water, even in the presence of a base, while SHM derivatives were so-

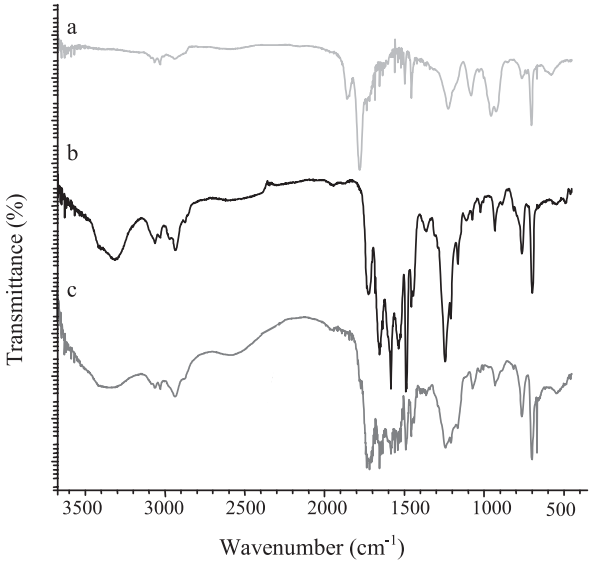


Fig. 1. Comparison of IR spectra of: a) SMA (**4**), b) SMAC-EDA-Fen (**5a**) and c) SHM-EDA-Fen (**6a**).

luble in aqueous basic media and methanol. Better hydrosolubility of conjugates **6a** and **6b** was assured by less extensive drug loading and the presence of free hydroxyl groups.

CONCLUSIONS

Several polymer-drug conjugates of fenoprofen and gemfibrozil were synthesized as potential macromolecular produgs: SMAC-EDA-Fen (**5a**), SMAC-EDA-Gem (**5b**), SHM--EDA-Fen (**6a**) and SHM-EDA-Gem (**6b**). Preliminary results showed that the prepared polymer-drug conjugates underwent hydrolysis and released the bound fenoprofen and gemfibrozil. However, detailed kinetic studies of chemical and potential enzyme hydrolysis still remain to be done.

Acronyms, abbreviations, symbols. – Bt – *N*-1-benzotriazolyl; BtH – benzotriazole; DMF – *N*,*N*-dimethylformamide; EDA – ethylenediamine; Fen – fenoprofen residue without carboxylic group; Gem – gemfibrozil residue without carboxylic group; NSAID – non--steroidal anti-inflammatory drug; SHM-EDA-Fen – [(styrene-2-hydroxyethyl maleamide)-(styrene-maleic acid) copolymer]-ethylenediamine-fenoprofen conjugate; SHM-EDA--Gem – [(styrene-2-hydroxyethyl maleamide)-(styrene-maleic acid) copolymer]-ethylenediamine-gemfibrozil conjugate; SMA – styrene-maleic acid anhydride copolymer; SMAC-EDA-Fen – (styrene-maleic acid copolymer)-ethylenediamine-fenoprofen conjugate; SMAC-EDA-Gem – (styrene-maleic acid copolymer)-ethylenediamine-gemfibrozil conjugate; TEA – triethylamine.

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S A Ž E T A K

Sinteza konjugata fenoprofena i gemfibrozila s kopolimerom stirena i maleinske kiseline

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U radu je opisana sinteza polimer-lijek konjugata polazeći od kopolimera stirena i anhidrida maleinske kiseline (SMA) i fenoprofena, odnosno gemfibrozila, ljekovitih tvari s kratkim vremenom zadržavanja u plazmi. Fenoprofen i gemfibrozil su prvo prevedeni u 2-aminoetilamide, koji su zbog slobodne amino skupine mogli reagirati s anhidridnim prstenovima u SMA. Modifikacijom tipa i vrste supstitucije pripravljeni su lipofilni i hidrofilni konjugati. Udio vezanog lijeka u konjugatima bio je između 17 and 47%.

Ključne riječi: fenoprofen, gemfibrozil, polimer-lijek konjugati, makromolekularni prolijekovi, kopolimer stirena i anhidrida maleinske kiseline

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