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Polymer conjugates with potential biological activity based on new derivatives of 2-mercaptobenzoxazole-synthesis and characterization

Research Article

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Abstract: New potentially biologically active compounds derived from 2-mercapto-benzoxazole were synthesized and coupled on polymeric support of poly (maleic anhydride-*alt*-vinyl acetate) for the preparation of polymer-drug conjugates with controlled drug release. All compounds were characterized by elemental and spectroscopy (FT-IR, ^{1}H-NMR) analysis. The toxicological tests recommend the products for further laboratory screening.

Keywords: *2-mercapto-benzoxazole derivatives • Hydrazides • Drug-polymer conjugates • Poly (maleic anhydride-alt-vinyl acetate)*

1.Introduction

The presence of the benzoxazole ring in numerous naturally occurring organic compounds (*i.e.*, enzymes, nucleic acids, vitamins, *etc.*), which has important therapeutic effects stimulated research in obtaining new benzoxazole derivatives, more intensely after 1964, when Harris and Folkers prepared vitamin B_6 starting from a 4, 5-disubstituted oxazole derivative. During the research evolution of these types of compounds, some are worth mentioning, such as Flunoxaprofen, a non-steroidian anti-inflammatory drug with important applications in modern chemotherapy [1]. Also, the role of some benzoxazole derivatives in inhibiting topoisomerase I and reverse-transcriptase HIV-1 was found to be of extreme importance in the chemistry of vital processes [2].

2-mercaptobenzoxazole and its derivatives are heterocyclic compounds of strong nucleophilic character with numerous applications in medicine, such as in the treatment of schizophrenia (4-7 substituted derivatives of arylsulfonyl benzoxazole [3]), Alzheimer (5, 6 substituted derivatives of 2-(4-dimethylaminophenyl)-1,3-benzoxazole [3,4]), diseases of the central nervous system [3], HIV (3H-pyrido-[2,1-b]-benzoxazole; 2-(4-amino-3-substituted-2-thioxo-2,3-dihydrotiazole-5-yl) benzoxazole [5]), cancer (isosteric benzoxazole [6]; conjugates based on [2,1-c] pyrrolo-benzoxazole [1,4]; benzodiazine [7,5]), tuberculosis (2-substituted, 5,7-di-tert-butyl-benzoxazole [4]).

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Thus, the first aim of this paper is the synthesis and characterization of new 2-mercaptobenzoxazole derivatives following both novel and classic methods and the evaluation of their acute toxicity.

In the last decade, the term of nanomedicine has been more and more used by physicians, biologists and scientists activate in human health research. Science Citation Index (Institute for Scientific Information, Thompson, Philadelphia, USA) reported the first publications mentioning this term around the year 2000. As a unanimously accepted concept, nanomedicine is considered as the use of nanoscale or nanostructured materials in medicine that, according to their structure, have unique medical effects, for example, the ability to cross biological barriers or the passive targeting of tissues. This definition does not include traditional small-molecule drugs as they are not specifically engineered on the nanoscale to achieve therapeutic effects that relate to their nanosize dimension [8-10]. However, the term refers to macromolecular drugs, respectively polymers with biological activity *per se* or polymers which attain biological effect by chemical binding of certain low molecular compounds on the main chain, thus, justifying the already popular term of "polymer therapeutics" (rational design of nanomedicines).

This type of drug-polymer associations, achieved by chemical binding of biologically active sites to macromolecular support are also called polymer-drug conjugates, a term first mentioned by Ringsdorf in 1975 [11]. According to his concept and the model proposed by Ringsdorf, a polymer-drug conjugate has triple structure: a support of macromolecular chain, containing reactive functional groups (or groups to be further functionalized) on which the biologically active molecule is bound through a spacer (generally, biodegradable in order to determine the release of the drug and having pending reactive groups-amine, carboxyl, hydroxyl, *etc.*), a strong hydrophilic group to ensure water solubility of the complex macromolecular architecture and a functional group able to be recognize by the cell receptors specific for the targeted organs. Of course, the above model is an ideal one, so for many versions being realized, sometimes have no functional groups to target to a certain organ. In the last two decades, the research in the field of drug-polymer conjugates has shown a real increasing interest [12], with an impressive number of papers being published [13-18].

Besides the well known advantages of using the drug-polymer conjugates for the treatment of certain diseases, the second aim of the paper was the preparation of such systems by chemical binding of the synthesized 2-mercaptobenzoxazole derivatives to a reactive, nontoxic and biocompatible macromolecular support, with biological activity of its own- poly(maleic anhydride-*alt*-vinyl acetate) [19]. Besides its biomaterial characteristics, the copolymer is highly reactive in normal temperature conditions (due to anhydride ring), without the need of a catalyst or high temperature and forms strong hydrophilic groups (-COOH), of the drug binding to the support. Thus, the present paper aims to synthesize new 2-mercaptobenzoxazole derivatives and their corresponding conjugates with poly(maleic anhydride-*alt*-vinyl acetate) and evaluate their physico-chemical characteristics and biological properties.

2. Experimental procedure

2.1. Material

2-Mercaptobenzoxazole was provided by Merck, ethyl chloroformate, ethyl chloroacetate, ethyl p-aminobenzoate, chloroacetyl chloride, sodium, ethyl acetate from Aldrich, dioxane from Fluka, acetone, anhydrous ethanol from Chemical Company S.A. The chemical substances were used as provided, without any supplementary purification.

2.2. Methods

Ethyl ester of benzoxazolyl-2-mercaptoformic acid (II) was obtained starting from 2-mercaptobenzoxazole and ethyl chloroformate in anhydrous ethanol, according to a method described in literature [20].

Cream coloured solid: (57.41 g, 87.2%), m.p. 85-87°C. IR; ν(cm^{-1}): 2850, 3300 (CH), 1730 (C=O), 745 (-C-S-). ^{1}H-NMR (DMSO-d$_6$, 400 MHz), δ (ppm): 1.6 (t, 3H, CH$_3$); 4.4 – 4.5 (d, 2H, CH$_2$); 7.40 (d, 2H Ar CH); 7.70 (d, 2H, Ar CH). Anal.calcd. for $C_{10}H_9NO_3S$ (%): C, 53.81; H, 4.03; N, 6.27; S, 14.39; Found: C, 54.20; H, 4.47; N, 6.69; S, 14.76.

Ethyl ester of benzoxazolyl-2-mercaptoacetic acid (III) was synthesized from 2-mercaptobenzoxazole and ethyl chloroacetate in anhydrous ethanol [20].
Grey solid: (19.1 g, 80.6%), m.p. 47 – 48 °C. IR; ν (cm^{-1}): 2933, 3000 (CH), 1728 (C=O), 744 (-CH$_2$-S-). ^{1}H-NMR (DMSO-d$_6$, 400 MHz), δ (ppm): 1.20 (t, 3H, CH$_3$), 3.4 – 3.7 (d, 2H, CH$_2$), 4.50 (S, 2H, CH$_2$S), 7.30 (d, 2H Ar CH), 7.60 (d, 2H, Ar CH). Anal. Calcd. for $C_{11}H_{11}NO_3S$ (%): C, 55.69; H, 4.64; N, 5.90; S, 13.5; Found: C, 56.10; H, 5.13; N, 6.37; S, 13.90.

Chloroacetyl-2-mercaptobenzoxazole (IV) was prepared by the reaction of 2-mercaptobenzoxazole sodium salt with chloroacetyl chloride in acetone [22].

Cream coloured solid: (1.78g, 78.24%), m.p. : 115-118°C. IR; ν (cm^{-1}): 2944 (CH), 1728 (C=O), 824 (C=N), 733 (C-Cl), 787 (-C-S-). ^{1}H-NMR (DMSO-d$_6$, 400 MHz),

δ (ppm): 2.78–4.08 (d, 2H, CH_2); 7.20 (d, 2H Ar); 7.30 (d, 2H, Ar); Anal.calcd. for $C_9H_6NO_2SCl$(%): C, 47.48; H, 2.65; N, 6.15; S, 14.08; Found:C, 47.29; H, 2.77; N, 6.26; S, 14.25.

UV spectrum in ethanol presents 2 absorption peaks at 255 nm and 298 nm (very intense), respectively.

Hydrazide of benzoxazolyl-2-mercaptoformic acid (V) was obtained by treating compound (II) with hydrazine hydrate in absolute ethanol [20].

White solid: (1.92 g, 92%), m.p. 168–171°C. FT-IR; v_{max} cm^{-1}: 3289 (NH), 1659 (CO-NH), 614 (-C-S-), 1508 (C=N). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ(ppm): 4.50 (s, 2H, NH_2), 7.20 – 7.40 (d, 2H, Ar CH), 7.50 – 7.9 (d, 2H, Ar CH), 9.5 (s, 1H, NH). Anal. Calcd. for $C_8H_7N_3O_2S$ (%): C, 45.93; H, 3.34; N, 20.09; S, 15.31; Found: C, 46.20; H, 3.65; N, 20.55; S, 15.43.

Hydrazide of benzoxazolyl-2-mercaptoacetic acid (VI) was synthesized by treating compound (III) with hydrazine hydrate in absolute ethanol [20].

White solid: (1.62 g, 72.65%), m.p. 173 – 175°C. FT-IR; v_{max} cm^{-1}: 3202, 3304 (NH), 2994, 3037 (CH), 1645 (-CO-NH-), 1503 (C=N). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.10 (s, 2H, NH_2), 4.37 (s, 2H, CH_2-S), 7.32 – 7.48 (d, 2H, Ar CH), 7.60 – 8.0 (d, 2H, Ar CH), 9.48 (s, 1H, NH). Anal.Calcd. for $C_9H_9N_3O_2S$ (%): C, 48.43; H, 4.03; N, 18.83; S, 14.34; Found: C, 48.82; H, 4.22; N, 19.27; S, 14.67.

Benzoxazolyl-2-mercapto-carboxymethyl-hydrazide (VII)

Chloroacetyl-2-mercaptobenzoxazole (IV) (0.004 mol) are suspended in dioxane (10 mL) and hydrazine hydrate (0.012 mol) and triethylamine (0.4 g) are added under stirring, obtaining a cream coloured precipitate. The stirring is continued for 4 hours, at room temperature, then the product is vacuum filtered and purified by recrystallization from boiling water.

Light cream solid: (0.33 g, 36.26 %), m.p. 190-192°C. FT-IR; v_{max} cm^{-1}: 2944 (CH), 1728 (C=O), 824 (C=N), 733 (C-Cl), 787 (-C-S-). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.78 – 4.08 (d, 2H, CH_2); 7.20 (d, 2H Ar). Anal. Calcd. for $C_9H_6NO_2SCl$ (%): C, 42.47; H, 2.63; N, 6.15; S, 14.06. Found: C, 43.08; H, 2.56; N, 6.07; S, 14.45.

p-Aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoformic acid (VIII)

Compound (V) (0.001 mol) is dissolved in dioxane (10 mL) then, separately, in ethyl p-aminobenzoate (0.002 mol) in dioxane (10 mL). The two solutions are introduced in a flask provided with ascendant condenser and kept under refluxion on a water bath for 2 hours. The, dioxane is evaporated under reduced pressure, thus obtaining a cream coloured precipitate.

Cream coloured solid: (0.214 g, 65.32%), m.p. 127 – 132°C. FT-IR; v_{max} cm^{-1}: 3329, 3420 (NH), 2983, 3281 (CH), 1595 (-CO-NH-), 1495 (C=N), 1680 (C=O). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.14-4.28 (d, 2H, NH_2), 6.55-6.56 (d, 2H, Ar CH), 7.18-7.26 (d, 4H, Ar CH), 7.58 – 7.60 (d, 2H, Ar CH), 7.89 – 7.98 (s, 1H, NHCO), 10.04 (s, 1H, NHCO). Anal.Calcd. for $C_{15}H_{12}N_4O_3S$ (%): C, 54.87; H, 3.65; N, 17.07; S, 9.75; Found: C, 54.30; H, 3.23; N, 17.28; S, 9.67.

p-Aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoacetic acid (IX) was synthesized in similar manner as compound (VIII), starting from compound (VI) (0.002 mol) and ethyl p-aminobenzoate (0.002 mol), each dissolved in 10 mL dioxane (10 mL).

Light cream solid: (0.34 g, 50.74%), m.p. 172-173°C. FT-IR; v_{max} cm^{-1}: 3304 (NH), 3000 (CH), 1642 (C=O), 1454 (C=N), 1536 (-CO-NH-). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 4.17-4.19 (d, 2H, CH_2), 4.20 – 4.22 (d, 2H, NH_2), 6.55 – 6.58 (d, 2H, Ar CH), 7.31 – 7.66 (m, 6H, Ar CH), 9.31 (s, 1H, NHCO), 9.72 (s, 1H, NHCO). Anal. Calcd. for $C_{16}H_{14}N_4O_3S$ (%): C, 56.14; H, 4.09; N, 16.37; S, 9.35; Found: C, 56.49; H, 3.93; N, 16.62; S, 9.72.

Benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of p-aminobenzoic acid (X) was prepared following a similar method as compound (VIII). Thus, compound (VII) (0.001 mol) and ethyl p-aminobenzoate (0.002 mol) are dissolved in anhydrous dioxane (20 mL) and a light grey precipate is obtained and purified by recrystallization from boiling ethanol.

Light grey solid: (0.29 g, 84.79 %), m.p. 160-162°C. IR; v_{max} (cm^{-1}): 3306 NH), 3112 (CH, Ar), 2986 (CH), 1726 (CO), 1613 (NH), 1571 (C=C), 1494 (C=N), 1336 (C-O), 1212 (C-N), 750 (C-S). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 3.37 (s, 1H, NH), 3.63 (s, 2H, CH_2), 4.44 (s, 2H, NH_2), 6.36-6.62 (m, 3H, Ar), 7.03 (m, 3H,Ar), 7.26 (m, 1H, Ar), 7.52 (m, 1H, Ar), 8.25 (s, 1H, NHCO). Anal.Calcd. for $C_{16}H_{14}N_4O_3S$ (%): C. 56,14; H, 4.09; N, 16.37; S, 9.35. Found: C, 56.21; H, 3.98; N, 16.53; S, 9.60.

Poly (maleic anhydride-alt-vinyl acetate) poly(MAVA) (XI) with molecular weight of 100,000 g mol^{-1} (determined by viscosimetric method) was obtained by the co-polymerization of maleic anhydride with vinyl acetate at 80°C, in the presence of benzoyl peroxide [19].

Benzoxazolyl-2-mercaptoformyl-hydrazine-anylide of poly (maleic acid-alt-vinyl acetate) (XII)

Poly (maleic anhydride-*alt*-vinyl acetate) (0.0025 mol) and p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoformic (VIII) (0.0025 mol) are each separately dissolved in dioxane (15 mL). The two solutions are mixed together wth stirring in a round bottom flask. The reaction is carried out at 110-115°C, in an oil bath for 2 hours. A homogenous solution is obtained, and the excess of dioxane is removed by

distillation under reduced pressure. The final product is precipitated by repeated washings in anhydrous ether, filtered and dried at 50°C for 180 minutes.

Yellow solid: (1.005 g, 78.51%); m.p. 161°C. IR; ν_{max} (cm^{-1}): 3242 (NH), 1703 (CO din COOH), 1684 (C=O), 1540 (CO-NH), 1496 (C=N), 743 (C-S). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.26 (t, 3H, CH_3), 3.20 (d,2H,CH_2), 4.19 (s,H, CH), 6.55 (d, 2H, Ar), 7.24 (m, 4H, Ar), 7.46-7.62 (d, 2H, Ar), 7.89 (s, 2H, NHCO), 10.04 (s, H, NHCO), 11.079 (s, H, COOH). Anal.Calcd. for $C_{23}H_{20}N_4O_8S$ (%) N, 10.93. Found: N, 5.86.

Benzoxazolyl-2-mercaptoacetyl-hydrazine-anylide of poly (maleic acid-alt-vinyl acetate) (XIII) was prepared in a similar manner as compound (XII), from poly(maleic anhydride-*alt*-vinyl acetate) (0.0025 mol) and p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoacetic acid (IX) (0.0025 mol) in dioxane.

Orange solid: (0.831 g, 63.19%), m.p. 170°C. IR; ν_{max} (cm^{-1}): 3307 (NH), 1717 (CO din COOH), 1644 (C=O), 1537 (CO-NH), 1454 (C=N), 807 (C-S), 739 (CH_2-S). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.25-1.28 (t,3H,CH_3), 3.57 (d,2H,CH_2), 4.18 (s,H, CH), 4.29 (d,2H,CH_2), 6.55-6.57 (d, 2H, Ar), 7.33-7.73 (d, 6H, Ar), 7.94-8.35 (s, 2H, NHCO), 12.50 (s, H,COOH). Anal. Calcd. for $C_{24}H_{22}N_4O_8S$ (%) N, 10.64. Found N, 5.394.

Benzoxazolyl-2-mercapto-carboxymethyl hydrazine-anylide of poly (maleic acid-alt-vinyl acetate) (XIV) was prepared by a similar method as compound (XII), starting from poly(maleic anhydride-*alt*-vinyl acetate) (0.0025 mol) and benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of p-aminobenzoic acid (0.0025 mol) in dioxane.

Cream coloured solid: (0.70 g, 53.23%), m.p. 184°C. IR; ν_{max} (cm^{-1}): 3123-3313 (NH), 2989 (CH, Ar), 1712 (CO din COOH), 1613 (CO), 1517 (CO-NH), 1494 (C=N), 786 (C-S). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ (ppm): 1.23 (D,2H,CH_2), 1.91 (s, 1H, NH), 1.93 (t, 3H, CH_3), 3.37 (d, 2H, CH_2), 3.52 (d, 2H, CH_2), 4.203 (s, 1H, CH), 6.55 (d, 2H, Ar), 6.90 (d, 2H, Ar), 7.10 (d, 2H, Ar), 7.63 (d, 2H, Ar), 8.34 (s, 1H, NHCO), 9.10 (s, 1H, NHCO), 11.4 (s, 1H, COOH). Anal.Calcd. for $C_{24}H_{22}N_4O_8S$ (%) N, 10.64. Found N, 7.38.

2.3. Characterizations

FT-IR spectra were obtained using a FTIR spectrophotometer (ATR) Bruker Tensor-27 (KBr disk technique);

^{1}H-NMR analysis was performed on a Bruker DRX 400 spectrometer (5mm QNP probe; 1H/13C/31P/19F. The compounds were previously dissolved into DMSO at a concentration of aprox. 7 mg mL^{-1}. The spectra were run at room temperature.

Silicone Graphics Indigoz and elemental analysis was made using Exeter Analytical CE 440 elemental analyzer. The melting points of the obtained compounds were determined with a Mel-Temp melting point module, provided with digital thermometer.

2.3.1. Determination of toxicity

Acute toxicity of the synthesized compounds (V-X and XII-XIV) and copolymer [poly (MAVA)] was evaluated using white male mice of 20±2g each. The mice were kept under observation for 7 days at constant temperature, receiving habitual nourishment. Mice weighing was done every two days, eliminating from the test groups the animals which presented weight loss. Every group consisted of 6 animals. The tested compounds were administered intraperitoneally as aqueous suspensions stabilized by Tween 80; mice mortality was registered after 24 hours, 48 hours and 7 days.

2.3.2. Determination of tuberculostatic activity

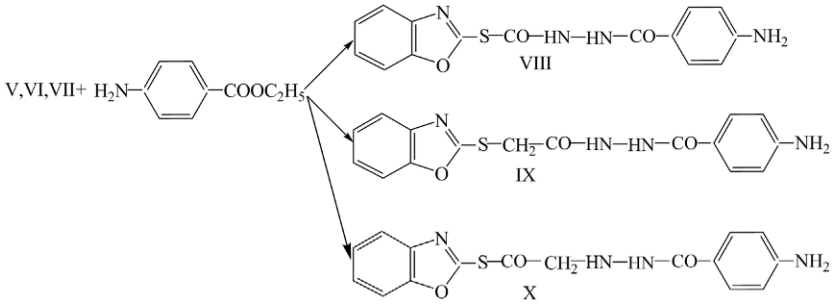
Compounds V-X and XII-XIV were tested for their *in vitro* biological activity, using the serial dilution technique, and *Youmans* medium with bovine serum as liquid environment, inoculated with *Mycobacterium tuberculosis*, var. *hominis* (strain $H_{37}R_v$), in a concentration of 10^{-2} mg mL^{-1} [20]. The biologically active compounds were previously dissolved in dimethylsulphoxide, due to their low solubility in saline solutions. Solutions formed of 100 µg compound dissolved in 1 mL solvent (DMSO/phosphate buffer solution pH 7 of ¼ (v/v)) were used in concentrations of 5, 10, 20, 30 and 40 µg compound/mL culture environment, the results being registered (Table 3) 6 and 15 days after inoculation.

3. Results and discussion

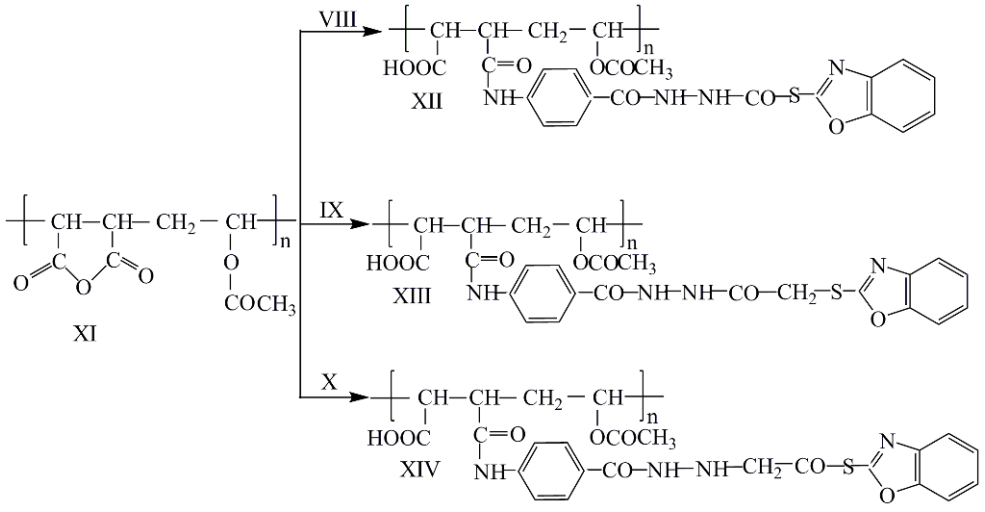
Compounds (VIII-X) were synthesized using as intermediates ethyl esters of benzoxazolyl-2-mercaptoformic (II), benzoxazolyl-2-mercaptoacetic (III) and chloroacetyl-2-mercaptobenzoxazole (IV), as well as their hydrazides (V-VII), which were treated with ethyl p-aminobenzoate (Scheme 1).

The chemical structure of compounds (VIII-X) was confirmed by elemental and spectroscopy analysis (FT-IR, ^{1}H-NMR). IR spectra for all evaluated compounds (VIII - X) present at 1454-1495 cm^{-1} an absorption band specific for C=N and at 1642-1680 cm^{-1} for C=O. The aromatic ring is highlighted by the presence of an absorption band at 3000-3281 cm^{-1}.

^{1}H-NMR spectra prove the presence of characteristic structural elements specific for compounds (VIII-X). Aliphatic and aromatic protons appear at



Scheme 1. Synthesis of p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoformic acid (VIII), p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoacetic acid (IX) and benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of p-aminobenzoic acid (X).



Scheme 2. Synthesis of benzoxazolyl-2-mercaptoformylhydrazine-anilide of poly (maleic anhydride-*alt*-vinyl acetate) (XII benzoxazolyl-2-mercaptoacetylhydrazine-anilide of poly (maleic anhydride-*alt*-vinyl acetate) (XIII) and benzoxazolyl-2-mercapto-carboxymethyl hydrazine-anilide of poly (maleic anhydride-*alt*-vinyl acetate) (XIV).

4.17-4.19 ppm and 6.55-7.66 ppm, respectively. NH_2 protons are shown at 4.14-4.28 and NH-CO protons (compounds VIII and IX) are proved by peaks at 7.89-10.04 ppm. In case of compound X, protons can be identified at 3.63 ppm-aliphatic, at 3.37 ppm-NH and at 4.44 ppm-NH_2. The two aromatic protons from the p-aminobenzoic ring are found at 6.36-6.62 ppm and the rest of the protons at 7.03-7.55. Amide protons are identified at 8.25 ppm.

Due to the fact that benzoxazole derivatives generally present important biological properties, one of the objectives of the paper was the grafting of the synthesized compounds (VIII-X) onto copolymer poly (maleic anhydride-*alt*-vinyl acetate) (XI) for the preparation of low toxic water soluble polymer-drug systems. The alkalinity of compounds (VIII-X) is rather high and can determine the opening of anhydride ring from copolymer (XI), thus resulting conjugates (XII-XIV) (Scheme 2).

By opening the anhydride ring, a new amidic derivative of maleic acid is formed; thus, the new synthesized conjugates are considered as derivative of poly (maleic acid-*alt*-vinyl acetate) (poly MAVA) and named as corresponding. The chemical structure of conjugates (XII-XIV) is confirmed by elemental (nitrogen content) and spectroscopic analysis (FT-IR, ^{1}H-NMR). FT-IR spectra show absorption peaks and bands for CO-NH at 1490-1495 cm^{-1}, for C=O (ester) at 1703-1717 cm^{-1} and for NH at 3242-3307 cm^{-1}, respectively. C=N group from oxazole heterocycle is shown by absorption bands at 1454-1496 cm^{-1}, C-S and CH_2-S at 743-807 cm^{-1} and 739 cm^{-1}.

^{1}H-NMR spectra present specific signal for aromatic rings at 6.55-7.73 ppm. CH_3 protons are marked by signals at 1.23-1.28 ppm (compounds XII, XIII; Fig. 1), at 1.93 ppm (compound XIV; Fig. 2), CH_2 protons at 3.20 ppm, (compound XII, CH-CH_2-CH), at 3.37 ppm (compound XIV) and at 3.57 ppm (compound XIII). The peak attributed to CH proton appears at 4.03 ppm (compound XIV), at 4.18 ppm (compound XIII) and at 4.19 ppm (compound XII) and CH_2 protons from the low molecular weight compounds appear at 3.53 ppm

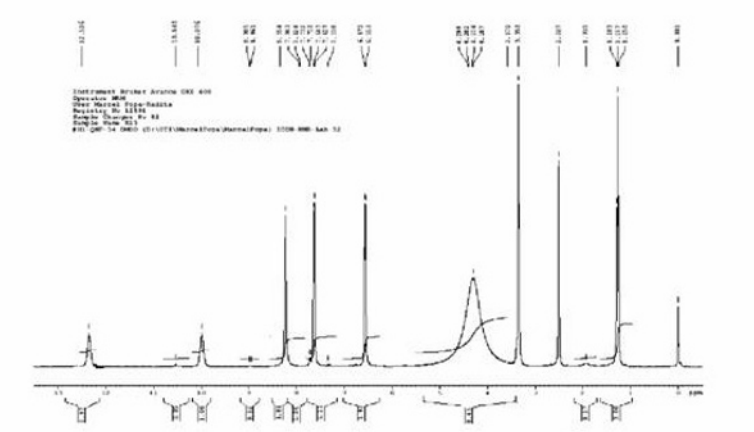


Figure 1. ^{1}H-NMR spectrum of benzoxazolyl-2-mercaptoacetylhydrazine-anilide of poly(maleic acid -*alt*-vinyl acetate) (XIII).

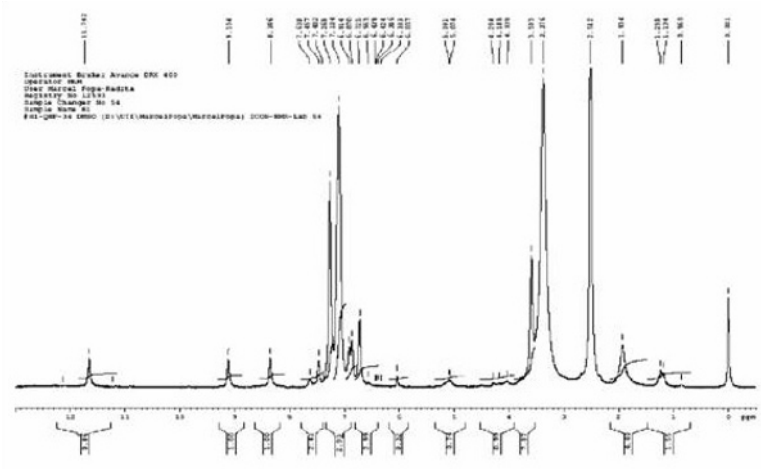


Figure 2. ^{1}H-NMR spectrum of benzoxazolyl-2-mercapto-carboxymethyl hydrazine-anilide of poly (MAVA) (XIV).

(compound XIV) and at 4.29 ppm (compound XIII), respectively. Specific peaks for amidic and carboxylic protons are shown at 7.89-10.04 ppm and 11.079-12.50, proving anhydride decyclization.

The yields of immobilizing biologically active compounds (XII- XIV) onto copolymer and the molar ratio between active principle and polymer support, calculated by elemental analysis for determining the nitrogen content are shown in Table 1.

The efficiency of coupling/immobilization of active compound on polymeric support is expressed as a ratio between the practical weight of benzoxazole derivative immobilized and the theoretical one (considering that all anhydride cycles reacted). Comparing the coupling efficiency of these compounds with other conjugates reported in previous work [20]: benzoxazolyl-2-mercaptocarbonyl-hydrazide of poly (MAVA) (66.38%), benzoxazolyl-2-mercaptoacetyl-hydrazide of poly (MAVA) (48.59%) and benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of poly (MAVA) (85.16%), lower yields seem to be obtained. This fact is probably due to the greater chain length of spacers, which increases the volume of the substitutes and determines important steric hindrance. Moreover, the water solubility of conjugates is different. If, benzoxazolyl-2-mercaptocarbonyl-hydrazide of poly (MAVA), benzoxazolyl-2-mercaptoacetyl-hydrazide of poly (MAVA) and benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of poly (MAVA) were found to be practically insoluble in water [20], by increasing the spacer chain length, the compounds (XII-XIV) gain water solubility, their behaviour being similar to Ringsdorf's model.

3.1. Biologic activity

3.1.1. Acute toxicity

If prepared conjugates are intended to be administered into an organism or in contact with it, they are required to present certain characteristics, among which biocompatibility and nontoxicity are essential. Lethal

dose 50 (LD_{50}) was determined using Spearman-Karber arithmetic method [21] and the results are presented in Table 2.

The results obtained from the biological tests reveal that poly(MAVA),the compounds (VIII-X), as well as their corresponding conjugates present lower toxicity, compared to the product previously reported [20], with a slightly higher toxicity shown by the polymeric support (Table 2). However, compared with isonicotinic hydrazide, the copolymer and the synthesized compounds are almost non-toxic, while the reference drug is moderately toxic (LD_{50} = 176). Therefore, the new compounds can be used for further laboratory screening.

Table 1. Coupling efficiency and molar ration between compounds (XII- XIV) and poly (MAVA).

Polymer-biologically active compound conjugate	Coupling efficiency (%; wt)	Active compound immobilized on poly MAVA (mol mol^{-1})
XII	53.9	0.313
XIII	50.7	0.263
XIV	69.4	0.436

Table 2. Acute toxicity for compounds VIII-X and XII- XIV.

Compound	LD_{50} (mg kg^{-1} body)			
	24 hours	48 hours	7 days	Average
XI	7053	7053	7002	7036
VIII	6840	6840	6795	6825
IX	6610	6610	6580	6600
X	6032	6032	6011	6025
XII	7370	7370	7310	7350
XIII	7036	7036	7003	7025
XIV	7118	7118	7064	7100

3.1.2. Tuberculostatic activity

The results obtained following the determination of the tuberculostatic activity are presented in Table 3.

Testing the *in vitro* biological activity of the investigated compounds against the development of Koch bacillus allowed the establishment of a correlation between their chemical structure and biological potential, as follows:

- Poly (MAVA) has no tuberculostatic potential (Table 3), but enables the biological activity of the immobilized compounds, through a new carboxylic group into the conjugate, appear following the decyclization of the anhydride ring; therefore, the drug-polymer conjugates present higher activity than the small molecules immobilized onto the polymer support

-The enhanced tuberculostatic effect of the conjugates (XII, XIII, XIV), compared to hydrazides (VIII, IX, X) is determined by the influence of the carboxy-anilid support of poly (MAVA) on the rest of benzoxazolyl-2-mercapto-hydrazide.

-The nature of the side chain from the structure of 2-mercapto-hydrazide derivatives immobilized onto carboxy anilid support of poly (MAVA) influences their biological activity. Thus, an increase of the tuberculostatic effect is shown for compound XIV, explained by the p-π conjugation present at a single CO-NH- group, in comparison with conjugates XII and XIII. This fact results in live organisms having an easier cleavage of benzoxazolyl-2-mercapto-carboxymethyl-hydrazide (X), responsable for the tuberculostatic activity, of similar

Table 3. Tuberculostatic activity of compounds VIII – XIV against *M. tuberculosis*, vs. isonicotinic hydrazide.

Compound	Active principle concentration in culture medium (μg mL^{-1})										MIC* (μg mL^{-1})
	5		10		20		30		40		
	6 days	15 days	6 days	15 days	6 days	15 days	6 days	15 days	6 days	15 days	
VIII	++	++	++	+	+	-	-	-	-	-	25
IX	++	++	++	++	+	+	-	-	-	-	30
X	++	++	++	++	+	+	+	-	-	-	35
XI	++	++	++	++	++	++	++	++	++	++	-
XII	++	++	+	+	-	-	-	-	-	-	20
XIII	++	+	+	-	-	-	-	-	-	-	15
XIV	++	+	-	-	-	-	-	-	-	-	10
Isonicotinic hydrazide	-	-	-	-	-	-	-	-	-	-	10

- no microbial growth; + medium microbial growth; ++ high microbial growth MIC = minimum inhibitory concentration*

degree as isonicotinic acid, comparing to the hydrazides of benzoxazolyl-2-mercaptoformic acid (VIII) and of benzoxazolyl-2-mercaptoacetic acid (IX).

4. Conclusions

There have been synthesized three new derivatives from mercaptobenzoxazole, respectively p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoformic acid (VIII), p-aminobenzoyl-hydrazide of benzoxazolyl-2-mercaptoacetic acid (IX) and benzoxazolyl-2-mercapto-carboxymethyl-hydrazide of p-aminobenzoic acid (X). The derivatives have been immobilized by amidic bonds onto poly(maleic anhydride-*alt*-vinyl acetate) to obtain new drug-polymer systems.

The chemical structure of the new compounds (VIII-X, XII-XIV) has been confirmed by elemental and spectroscopic analysis (FT-IR, ^{1}H-NMR).

Acute toxicity of VIII-X and XII-XIV has been evaluated by registering LD_{50} and revealed that hydrazides immobilization on copolymer support determined the decrease of toxicity for compounds VIII-X. Also, LD_{50} values classify them as practically nontoxic, compared to compounds which do not contain ethyl p-aminobenzoate.

Also, the *in vitro* biological activity of hydrazides and conjugates against *Mycobacterium tuberculosis*, was evaluated with the conjugates presenting similar minimum inhibitory concentration as isonicotinic hydrazide.

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