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Synthesis of pro-prodrugs L-lysine based for 5-aminosalicylic acid and 6-mercaptopurine colon specific release

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ABSTRACT

The aim of this work is to design, prepare and characterize L-lysine based prodrugs capable of targeting 6-mercaptopurine to the colon, an anti-tumor and immunosuppressant drug, and 5-aminosalicylic acid (5-ASA), drug of choice for inflammatory bowel disease (IBD). More specifically, Nε-feruloyl-S-(6-purinyl)-L-lysine and Nε-acryloyl-S-(6-purinyl)-L-lysine were synthesized and then characterized by FT-IR, ^{1}H-NMR and GC/MS spectroscopies. The ability of feruloyl derivative in inhibiting lipid peroxidation in rat liver microsomal membranes, induced in vitro by *tert*-butyl hydroperoxide as source of free radicals, was evaluated. Moreover, Nε-acryloyl-S-(6-purinyl)-L-lysine, polymerizable prodrug, was used to microspheres realization for 5-ASA release. These lasts, obtained by emulsion inverse technique, were characterized by light scattering and scanning electron microscopy (SEM) analysis. The microspheres equilibrium swelling degree was evaluated and showed good swelling behaviour in simulating colonic fluids. Results confirm the possibility that the application range of L-lysine prodrug can be extended to the treatment of intestinal diseases whose conventional therapy envisages medications with serious side effects that, thanks to this new strategy, can be minimized in an optimal way.

1. Introduction

During the last few decades, the pharmaceutical research has focused its attention on the development of formulations that can release the drug in the body in a controlled and specific manner to decrease the number of daily dosing, improve patient compliance and minimize adverse reactions. These formulations, defined as controlled release systems or Drug Delivery System outweigh the disadvantages associated with conventional treatment systems.

The creation of pharmaceutical forms able of selective colon release is gaining particular interest, above all, for the treatment of local diseases such as ulcerative colitis, Crohn's disease, rectal cancer, and irritable bowel syndrome (Friend, 2005; Gazzaniga et al., 2006; Jain et al., 2007).

A prodrug should be sufficiently hydrophilic and bulky to minimize the absorption in the stomach and small intestine, but must become more lipophilic in the colon to be absorbed. Due to the wide variety of enzymes produced by intestinal flora at this level, generally anaerobic bacteria responsible of different enzymes production such as esterases, amidase, nitroreductase, azoreductase, deaminase, urea dehydroxylase, it has been possible to prepare prodrugs subject to enzymatic cleavage (Kinget et al., 1998). Particular interest in recent years has been focused on the activity of azoreductase that catalyzes the reduction of a diazo bond with regeneration of two primary amino groups and glycosidases that catalyzes the hydrolysis of glycosidic bonds. The colon-specific prodrug for excellence, presenting a diazo bond, is sulfasalazine; however, other prodrugs were developed by coupling, through a β-glycosidic bond, an hydrophilizing residue (glucose or galactose) to steroids such as dexamethasone, prednisolone, hydrocortisone and fluorocortisone (Van den Mooter, 2006). The sugar presence makes the therapeutic agent sufficiently polar to suffer minimal absorption in the small intestine, while in the colon, thanks to the bacterial glycosidases activity, the polar fraction releases the steroid, which can carry out its action (Rubinstein, 2005; Makins and Cowan, 2001; Taxonera et al., 2009). The prodrugs based on amino acids are very effective in the treatment of IBD (inflammatory bowel disease) for the presence of very chemically reactive groups that are, at the same time, sites of various enzymes action (esterase, amidase, azoreductase); in particular, L-lysine possesses three sites of functionalization, two amino groups and a carboxyl one. For this reason, a pro-prodrug L-lysine based, binding 5-ASA and *trans*-ferulic acid as active substances was recently prepared in order to optimize biocompatibility and allow the drug colon-specific release (Cassano et al., 2009). Functionalization occurs at the level of the amino group in ε position and the amino group directly linked to the chiral carbon with the formation of amide or diazo bonds. The presence of these multiple sites allowed us to

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Table 1
Reaction conditions for **3a** and **3b** synthesis.

Reaction conditions for **3a**			Reaction conditions for **3b**		
Reagent	Amount (g or ml)	mmol	Reagent	Amount (g or ml)	mmol
MKC	8.30	0.085	FC	16.51	135.5
2a	0.065	0.305	**2b**	0.308	0.954
DCC	0.070	3.4×10^{-4}	DCC	0.220	1.051
DMAP	0.004	0.030	DMAP	0.012	0.095
6-MP	0.208	1.220	6-MP	0.650	3.816

design pro-prodrugs for the site-specific release of various pharmacologically active molecules. In particular, objective of this study, was the realization of both pro-prodrugs L-lysine based containing acrylic moieties and mercaptopurine or *trans*-ferulic acid and mercaptopurine. This latter was submitted to antioxidant activity evaluation. On the other hand, the polymerizable derivative was employed to prepare microspheres useful as carrier of other colon specific drugs and their swelling and release behaviour were also evaluated.

2. Materials and methods

2.1. Materials

All solvents of analytical grade, were purchased from Carlo Erba Reagents (Milan, Italy): acetone, chloroform, dichloromethane, ethanol, ethyl ether, isopropanol, methanol, N,N-dimethylformamide (DMF), n-hexane and tetrahydrofuran (THF). N-hexane, chloroform, N,N-dimethylformamide (DMF) and tetrahydrofuran were purified by standard procedures. Monohydrochloride L-lysine (MW = 182.65, purity ≥ 98%), 6-mercaptopurine monohydrate (MW = 170.19, purity 98%), methacryloyl chloride, basic copper carbonate, KOH (pellets), 8-quinolinol, sorbitan trioleate (Span 85), polyoxyethylene sorbitan trioleate (Tween 85) N,N,N′,N′-tetramethylethylenediamine (TMEDA), N,N-dimethylacrylamide (DMAA), ammonium persulfate (APS), dicyclohexylcarbodiimide (DCC), dimethylaminopyridine (DMAP) and thionyl chloride ($SOCl_2$) were purchased from Sigma–Aldrich (Sigma Chemical Co, St. Louis, MO, USA).

2.2. Methods

The infrared spectra were obtained from KBr pellets using a FT-IR spectrometer Perkin-Elmer 1720, in the range 4000–400 cm^{-1} (number of scans 16). ^1H NMR spectra were processed using a spectrometer Burker VM30; chemical shifts are expressed in δ and referred to the solvent. The structures of the compounds synthesized were confirmed also by GC–MS Hewlett Packard 5972. UV–vis spectra were realized through a UV-530 JASCO spectrophotometer. The light scattering was performed with a Brookhaven 90 plus particle size analyzer. The samples were lyophilized utilizing a "freezing–drying" Micro module apparatus, Edwards. Scanning electron microscopy (SEM) photographs of the microspheres were obtained with a JEOL JSMT 300 A; the surface of the samples was made conductive by deposition of a gold layer on the samples in a vacuum chamber.

2.3. Synthesis of prodrugs Nε-acryloyl-S-(6-purinyl)-L-lysine (*3a*) and Nε-feruloyl-S-(6-purinyl)-L-lysine (*3b*)

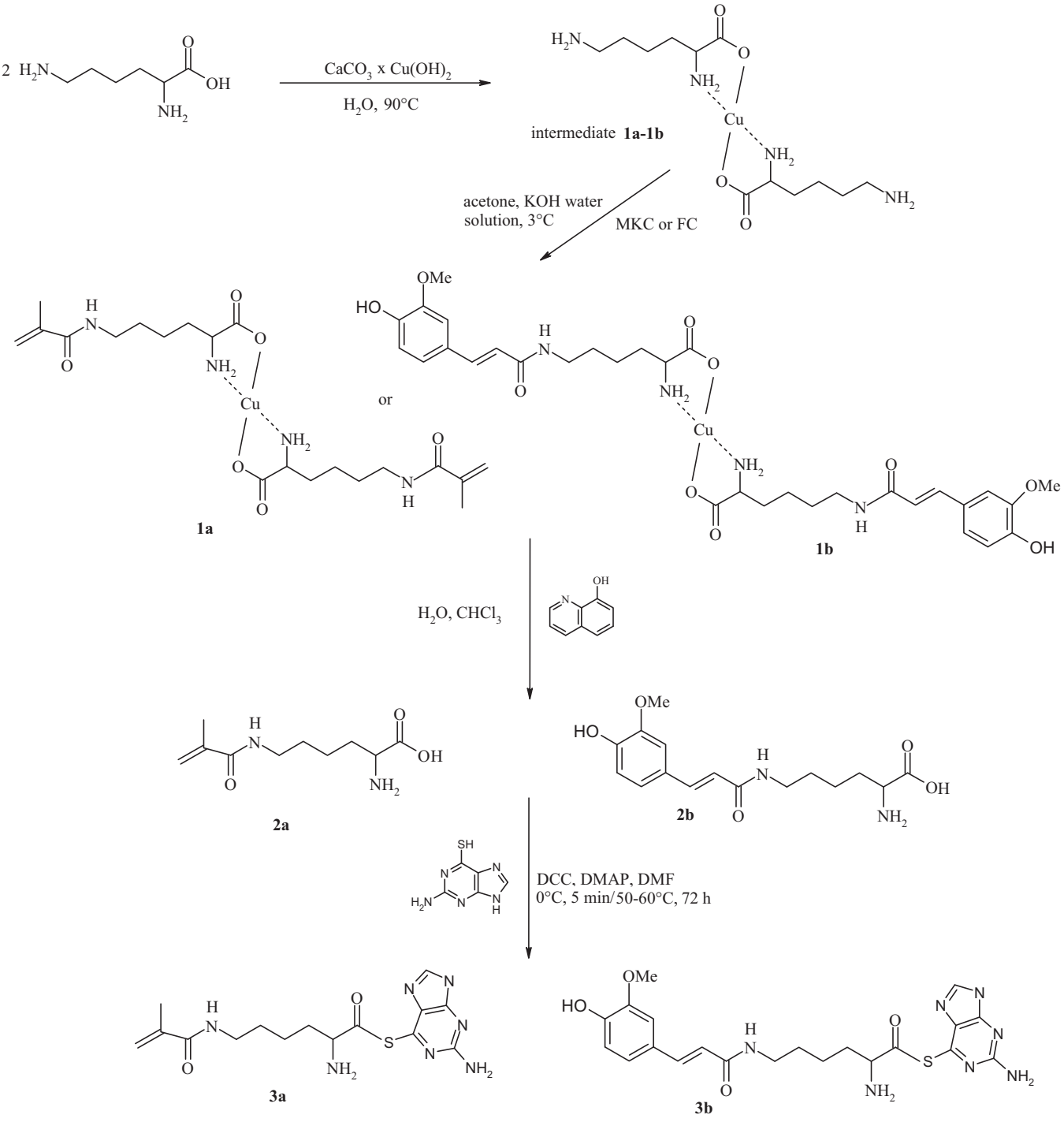
The reaction was conducted according to the procedure reported in the literature (Sinha and Kumria, 2001). Briefly, 50 g (274 mmol) of monohydrochloride L-lysine were dissolved in 600 ml of distilled water and the reaction mixture was left under reflux and magnetic stirring until the temperature reached a value of 90 °C. Then, 33.3 g (269 mmol) of copper carbonate ($CuCO_3$) were added slowly to obtain intermediate **1a**–**1b** (Scheme 1). Subsequently, 290 ml (3.945 mmol) of acetone and a potassium hydroxide aqueous solution (15.37 g, 274 mmol of KOH in 137 ml of distilled water) were added, under stirring still. Then, methacryloyl chloride (MKC) or *trans*-ferulic acid acyl chloride (FC) prepared as described in our previous work (Cassano et al., 2009) (Table 1) were added and temperature given to 3 °C. Then, a potassium hydroxide aqueous solution (17.26 g, 308 mmol of KOH in 154.8 ml of distilled water) was added for four times every 5 min and the reaction was left for 12 h. After that, the obtained precipitate was filtered and washed with distilled water, methanol and diethyl ether, drying the product after each washing. Obtained products (**1a**–**1b**) were blue colored and analyzed though FT-IR spectroscopy (Table 2). Yield: **1a** 78%; **1b** 81%.

Afterwards, 10 g (21 mmol) of products **1a**–**1b** were dissolved in 138 ml of distilled water, then a solution of chloroform containing the decomplexing agent, 8-quinolinol (3.64 g, 25 mmol of 8-quinolinol dissolved in 138 ml of chloroform), was added and the reaction left to reflux for more than 12 h under stirring. Extraction with chloroform furnished an aqueous solution containing decomplexed products (product **2a**–**2b**) that were purified through recrystallization in THF and characterized by FT-IR, GC/MS and ^1H NMR spectroscopies (Table 2). Esterification of product **2** led to the desired pro-prodrug. Yield: **2a** 94%; **2b** 88%

The reaction was conducted according to the procedure reported in the literature (Matthew et al., 2007). Products **2a**–**2b** (Table 1) were dissolved in the minimum amount of dimethylformamide (DMF) previously purified. After dissolution, dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) were added (Table 1) and the reaction mixture was brought to reflux at 0 °C and left for 5 min under stirring. Then, 6-mercaptopurine (6-MP) was slowly added dropwise (Table 1) after its dissolution in the minimum amount of DMF. The reaction was carried out with stochiometric ratio 1:4 for derivatized L-lysine and 6-mercaptopurine, respectively. The system was left under reflux for 72 h at the temperature of 50–60 °C. The reaction was constantly monitored by TLC. Finally, DMF was removed by distillation and the crude product was washed with methanol, to remove dicyclohexylurea (DCU), then dried and purified by chromatographic column. The obtained product (**3a**–**3b**) was carefully analyzed by GC/MS and NMR spectrometry (Table 2). Yield: **3a** 65%; **3b** 70%.

2.4. Preparation of microspheres Nε-acryloyl-S-(6-purinyl)-L-lysine (*3a*) based

A round-bottomed cylindrical glass reaction vessel fitted with an anchor-type stirrer of suitable capacity (100–150 ml), was flamed in a nitrogen flow and after cooling immersed in a bath thermostatically controlled at 40 °C. Dry n-hexane (20 ml) and chloroform (18 ml) were introduced as dispersant phase, then kept under mechanical agitation for 30 min. Polymerization reaction was conducted in according to the procedure reported in literature (Freiberg and Zhu, 2004). A fixed amount (42 mg,



Scheme 1. Synthetic routes for **3a** and **3b**.

Table 2
FT-IR, GC/MS and ^{1}H NMR data.

Product	*M*/*Z*	Wavenumber ν (cm^{-1})	Chemical shift (δ) ppm
2a	55 (100%)	1657 (–CONH), 1617 (–COOH), 917 (C=CH_2)	(D_2O) 5.40 (1H, sb), 5.16 (1H, sb), 3.33 (1H, m), 2.76 (2H, t), 1.66 (3H, s), 1.08–1.60 (6H, m)
3a	55 (7%), 69 (100%), 367 (2%)	1735 (–COSR), 1651 (–CONH), 916 (–C=CH_2)	(D_2O) 8.66 (1H, s), 8.46 (1H, s), 5.53 (1H, sb), 5.17 (1H, sb), 3.45 (1H, sb), 3.01 (2H, m), 2.10 (3H, s), 1.50–1.90 (6H, m)
2b	222 (100%), 77 (6%), 177 (71%)	1711 (–CONH)	(CD_3OD) 7.75 (1H, sb), 7.27 (2H, m), 6.85 (1H, d), 6.60 (1H, sb), 3.90 (3H, s), 3.36 (1H, m), 2.78 (2H, t), 1.12–1.72 (6H, m)
3b	55 (38%), 368 (100%)	1707 (–COSR), 1656 (–CONH)	(D_2O) 8.43 (1H, s), 8.25 (1H, s), 7.77 (1H, m), 7.63 (1H, m), 7.14 (1H, m), 6.88 (1H, m), 6.52 (1H, m), 3.70 (3H, s), 3.42 (1H, sb), 3.07 (2H, m), 1.50–1.90 (6H, m).

0.114 mmol) of **3** was dissolved in 4 ml of distilled water, sonicated for a few minutes to obtain a solution, then added with 7.21 μl (0.057 mmol) of the comonomer N,N-dimethylacrylamide (DMAA) and 800 mg of ammonium persulfate which acts as an initiator radical.

The density of the organic phase was adjusted by the addition of the two organic solvent so that the aqueous phase sank slowly when stirring stopped. After that, it was first added of sorbitan trioleate (Span 85, 150 μl) and polyoxyethylene sorbitan trioleate (Tween 20, 150 μl). After 10 min, 150 μl of N,N,N′,N′-tetramethyl diethylamine (TMEDA) were added and reaction was left under stirring for 3 h. Microspheres so obtained were filtered, washed with 100 ml portions of 2-propanol, ethanol, acetone and diethyl ether and dried overnight under vacuum. Their characterization was effected by optical microscopy and FT-IR spectroscopy.

2.5. Size distribution analysis

The size of microparticles was determined by dynamic light scattering (DLLS) using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation, New York, USA) at 25 °C by measuring the autocorrelation function at 90° scattering angle. Cells were filled with 100 ml of sample solution and diluted to 4 ml with filtered (0.22 mm) water. The polydispersity index (PI) indicating the measure of the distribution of nanoparticle population (Koppel, 1972) was also determined. Six separate measurements were made to derive the average. Data were fitted by the method of inverse "Laplace transformation" and Contin (Provencher, 1982a,b).

2.6. Swelling studies

The swelling behaviour was investigated in order to check the hydrophilic affinity of spherical microparticles. Typically, aliquots (50 mg) of dried materials were placed in a tared 5-ml sintered glass filter (Ø 10 mm; porosity G3), weighed, and left to swell by immersing the filter in a beaker containing the swelling media (acidic solution pH 1.2, simulated gastric fluid; phosphate buffer pH 6.5, simulated colonic fluid; phosphate buffer pH 7.8, simulated small intestinal fluid) (Khan et al., 1999). At predetermined times (1, 6, 12 and 24 h), the excess of water was removed by percolation and then the filter was centrifuged at 3500 rpm for 15 min and weighed. The filter tare was determined after centrifugation with only water. The weights recorded at different times were averaged and used to give the equilibrium swelling degree (*Wt*(%)) by Eq. (1) where *Ws* and *Wd* are the weights of swollen and dried microspheres, respectively. Each experiment was carried out in triplicate and the results were in agreement within ±4% standard error.

$$Wt\ \ (\%) = \frac{Ws - Wd}{Ws} \times 100 \qquad (1)$$

2.7. Incorporation of drug into preformed microspheres

Incorporation of drugs into microspheres was performed by soaking procedure as follows: 150 mg of preformed empty microspheres (prepared as described above) were wetted with 20 ml of water in a concentrated drug solution (0.002 mg/ml). After 3 days, under slow stirring at 37 °C, the microspheres were filtered and dried at reduced pressure in presence of P_2O_5 to constant weight (Pitarresi et al., 2004). The loading efficiency percent (LE, %) of all samples were determined by spectrophotometric analysis of filtered solvent in according to Eq. (2):

$$\text{LE}\ \ (\%) = \frac{C_i - C_o}{C_i} \times 100 \qquad (2)$$

Here C_i is the concentration of drug in solution before the loading study and C_o is the concentration of drug in solution after the loading study.

2.8. In vitro drug release from microparticles

Dried microspheres (10 mg) were dispersed in 6 ml of swelling media (acidic solution pH 1.2, simulated gastric fluid; phosphate buffer pH 7.8, simulated small intestinal fluid; phosphate buffer pH 6.5, simulated colonic fluid). The test tubes were maintained at 37 °C in an horizontal-shaking bath and shaked at a rate of 100 rpm. At predetermined intervals, the samples were centrifuged, 5 ml of supernatant was removed and the medium was replaced with fresh solutions to maintain the same total volume throughout the study. The concentration of 5-aminosalicylic acid was determined by UV spectrophotometry at fixed wavelengths depending on the different swelling media. In particular, the release profile in the acidic solution at pH = 1.2 was recorded at 204 nm (ε = 4566 l mol^{-1} cm^{-1}); the release profile in the phosphate buffer at pH 6.5 was recorded at 198 nm (ε = 2840 l mol^{-1} cm^{-1}); the release profile in the phosphate buffer at pH 7.8 was recorded at 196 nm (ε = 19 000 l mol^{-1} cm^{-1}). Each in vitro release study was repeated in triplicate. Drug release was calculated in terms of cumulative release (% drug release) (Khan et al., 1999).

2.9. Evaluation of Nε-feruloyl-S-(6-purinyl)-L-lysine (**3b**) antioxidant activity

In order to evaluate its antioxidant activity, product **6** was subjected to the malondialdehyde (MDA) test in rat liver microsomal membranes (Trombino et al., 2008). Liver microsomes were prepared from Wistar rats by tissue homogenisation with 5 volumes of ice-cold, 0.25 M sucrose, containing 5 mM HEPES, 0.5 mM EDTA, at pH 7.5, in a Potter–Elvehjem homogenizer (Tatum et al., 1990). Microsomal membranes were isolated by removal of the nuclear fraction at 8000 × *g* for 10 min and removal of the mitochondrial fraction at 18 000 × *g* for 10 min. The microsomal fraction was sedimented at 105 000 × *g* for 60 min, and the fraction was washed once in 0.15 M KCl and collected again at 105 000 × *g* for 30 min (Ohta et al., 1997). The membranes, suspended in 0.1 M potassium phosphate buffer, at pH 7.5, were stored at −80 °C. Microsomal proteins were determined by the Bio-Rad method (Bradford, 1976). The microsomes were suspended in 0.1 M phosphate buffer at pH 7.5. The complex **6** was quickly added to microsomes. Microsomes were added to the amount of *tert*-BOOH to reach a final concentration of 0.25 mM. Microsomal suspensions, gently suspended using a Dounce homogeniser, were incubated at 37 °C in a shaking bath under air and in the dark for 1 h. Thereafter, aliquots of 1 mL of microsomal suspension (0.5 mg of proteins) were mixed with 3 mL of 0.5% TCA and 0.5 mL of TBA solution (two parts 0.4% TBA in 0.2 M HCl and one part distilled water) and 0.07 mL of 0.2% BHT in 95% ethanol. Samples were then incubated in a 90 °C bath for 45 min. After incubation, the TBA–MDA complex was extracted with 3 mL of isobutyl alcohol. The absorbance of the extracts was measured by the use of UV spectrophotometry at 535 nm, and the results were expressed as nmol per mg of protein, using an extinction coefficient of 1.56×10^{-5} l m^{-1} cm^{-1}.

3. Results and discussion

Literature data concerning studies of carrier molecules useful in site-specific prodrugs preparation, show an increased interest toward these materials, because they combine the biocompatibility with the site-specificity against target organs. For these

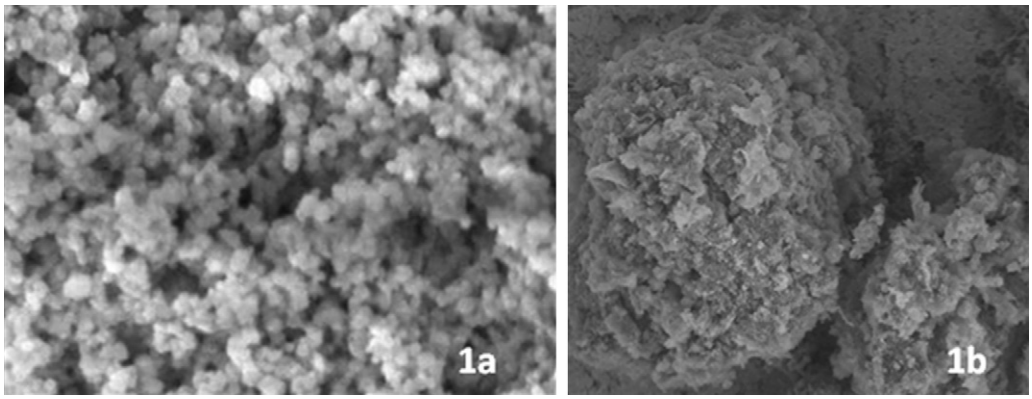


Fig. 1. SEM micrographs.

reasons, in the present work, we synthesized and characterized prodrugs L-lysine based. Due to the presence of three functionalizable groups (one carboxylic- and two amino-groups) L-lysine was derivatized both with 6-mercaptopurine, immunosuppressive and anticancer drug, and *trans*-ferulic or methacrylic acid. In addition, the methacrylic prodrug was polymerized with a specific comonomer to obtain microspheres able to incorporate other colon specific active substances. The microspheres swelling behaviour at different pH was evaluated with great results above all at simulating colonic fluids. Moreover the release profile of prepared microspheres was studied according to some studies concerning the variation of the pH value throughout the GI tract; in fact important investigations using sensitive and reliable equipments contradict the traditional view and provide evidence of a fall in pH at the GI region between ileum and colon. Apparently, the colon has a lower pH value (6.5) than the small intestine (7.0–7.8), and the jejunal region of some individuals has a higher pH (range 6.1–7.2) than the small intestine or colon of other individuals (Evans et al., 1988). On the other hand, the ferulate derivative was submitted to antioxidant activity evaluation.

3.1. Microspheres preparation and characterization

Chemical groups susceptible of radical polymerization were introduced onto L-lysine by acylation with MA. Under reaction condition only L-lysine ε-amino groups react with acylating agent. Microspheres were synthesized by copolymerization of Nε-acryloyl-S-(6-purinyl)-L-lysine (**3a**) with a comonomer such as DMAA. The reaction was started using TMEDA and ammonium persulfate as initiator system. The obtained materials were characterized by Fourier Transform IR spectrometry, particle size distribution analysis and morphological analysis. The FT-IR spectra of all samples shows the disappearance of bands at 944 and 934 cm^{-1} awardable to C–C double bond of methacrylic groups.

3.2. Particle sizes and scanning electron microscopy (SEM)

The light scattering analysis confirmed microparticle presence with a good polydispersity index (0.119) and an average diameter of 13 μm approximately.

Morphological analysis of the microspheres was carried out using SEM, which is the most widely used of the surface analytical techniques. SEM represents an invaluable tool for studying surface topography and failure analysis. The technique, which enables qualitative three dimensional (3-D) imaging of surface properties, clearly illustrated that the microparticles had a spherical shape. In Fig. 1a the spherical shape of our microspheres are evident. Fig. 1b shows a spongy surface characterized by a high degree of porosity.

3.3. Microspheres swelling behaviour

Investigation of the applicability of these microparticles in controlled release was done by studying their swelling behaviour. The value of contained water percentage was determined in aqueous media which simulates some biological fluids, such as gastric (pH 1.2), intestinal (pH 7.8) and colonic (pH 6.5) at 37 °C. The data reported in Table 3 illustrate the water uptake, in grams per grams of dry copolymer, for each composition and pH studied. The prepared materials show different water affinity at the different pH values. In particular, at pH 1.2 there is a considerable lowering of the water affinity due to acidic groups unionized at this pH value. When the pH is 6.5, the water content is greater than that found at pH 1.2. It is possible to explain this behaviour as a consequence of electrostatic repulsions between polymeric chains due to the increase of dissociated groups at pH 6.5. Microspheres showed a best swelling behaviour in solutions simulating colonic environment. With the time, this effects increase in an extraordinary manner, rather than to decrease with the maximum swelling effect at 24 h. These results are very encouraging and confirm the application of the prodrug in the colonic tract for the treatment of IBD.

3.4. Evaluation of the in vitro drug release from microparticles

In order to estimate the ability of prepared matrices to release encapsulated drug, the beads were loaded with 5-aminosalicylic acid by soaking procedure and the loading efficiency (LE, %) was determined by spectrophotometric analysis such as reported in experimental part. The experimental data, reported in Fig. 2, are interesting. The 5-aminosalicylic acid was almost completely loaded on the polymeric beads (LE (%) = 78.6). It is possible to explain this behaviour as consequence of strong interactions between polymeric matrix and acidic drugs, such as 5-ASA. Drug

Table 3
Microsphere swelling behaviour.

Time	Swelling (α %)		
	pH 1.2	pH 6.5	pH 7.8
1 h	270.2%	342.5%	284.3%
6 h	254.8%	691.9%	302%
12 h	273%	803%	616.2%
24 h	283%	1067%	841%

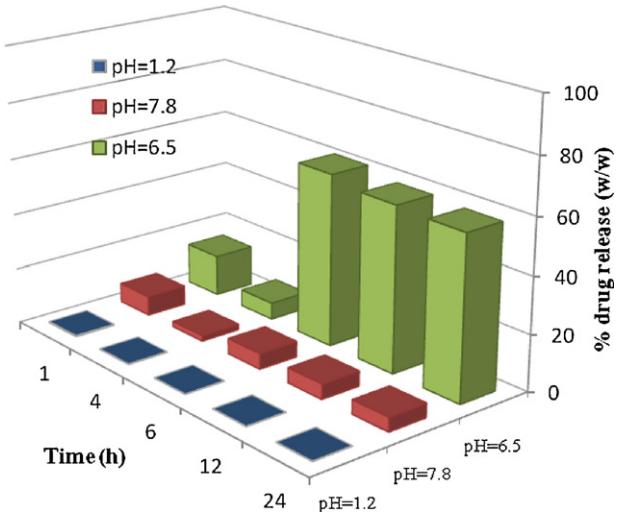


Fig. 2. 5-ASA release evaluation. (For interpretation of the references to color in this artwork, the reader is referred to the web version of this article.)

release profile was determined by spectrophotometric analysis. The drug release was expressed as the percent of drug delivered, related to the effectively entrapped total dose, as a function of time. We have carried out the in vitro release studies at 37 °C. The experimental data showed an increase of 5-ASA release at pH 6.5; the drug is released in very low amounts both in the stomach that in the small intestine.

3.5. Evaluation of Nε-feruloyl-S-(6-purinyl)-L-lysine (**3b**) antioxidant activity

The ability of **3b** to inhibit lipid peroxidation induced by a source of free radicals such as *tert*-BOOH, was examined in rat liver microsomal membranes over 120 min of incubation. In order to evaluate ferulate L-lysine and 6-MP antioxidant properties, the same experiment was performed. The following graph (Fig. 3) shows the complex **3b** lipid peroxidation inhibition in relation to ferulate lysine and 6-MP behaviour. It shows that 6-MP acts as a pro-oxidant agent while ferulate L-lysine and its purinic thioester present a strong antioxidant activity and a remarkable capacity to overthrow the pro-oxidant effect of 6-MP itself. The association of 6-MP and *trans*-ferulic acid has, therefore, a very important advantage: *trans*-ferulic acid counteracts the pro-oxidant action of 6-MP and limits, therefore, the side effects giving thus to our prodrug an important

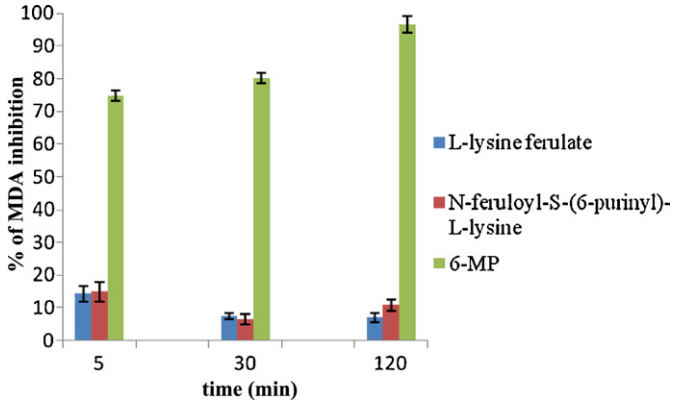


Fig. 3. Evaluation of Nε-feruloyl-S-(6-purinyl)-L-lysine (**3b**) antioxidant activity. (For interpretation of the references to color in this artwork, the reader is referred to the web version of this article.)

characteristic that allow to have a high potential in biomedical field minimizing adverse reaction of conventional therapies.

The effects of both pro-prodrug and L-lysine ferulate on the lipid peroxidation were time-dependent with the preservation of their antioxidant activity up to 2 h (Fig. 3).

4. Conclusions

Prodrugs L-lysine based containing purinic moieties were successfully obtained and characterized by common spectroscopy techniques as FT-IR, GC/MS and ^{1}H NMR. Using polymerizable or antioxidant substances two prodrugs were prepared. The polymerizable one was used to prepare microspheres useful as carrier of other colon specific drugs like 5-ASA. Scanning electron microscopy showed a spherical shape and a spongy surface. The light scattering technique allowed to effect dimensional analysis. Microspheres swelling studies were also conducted and showed a very good swelling behaviour in simulating colonic fluids. On the other hand, the ability of feruloyl derivative in inhibiting lipid peroxidation in rat liver microsomal membranes induced in vitro by *tert*-butyl hydroperoxide as source of free radicals was evaluated. Results revealed an important capacity to overthrow the intrinsic pro-oxidant effect of 6-MP. Finally, our prodrugs could have high potential in biomedical field, in particular in the tumors treatment, targeting 6-mercaptopurine to the colon and outweighing the disadvantages associated with conventional treatment systems.

Acknowledgement

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