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Polymeric prodrugs: α,β-poly (*N*-hydroxyethyl)-DL-aspartamide as a macromolecular carrier for some non-steroidal anti-inflammatory agents

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Summary

The properties as a drug carrier of the biodegradable polymer, α,β-poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) were investigated. Thus, naproxen- and 4-biphenylacetic acid-PHEA adducts were prepared and characterized; drug content was evaluated by both UV and elemental analysis and hydrolysis of the adducts. In vivo analgesic and anti-inflammatory tests indicated that the adducts retain an activity comparable to that of the free drugs.

Introduction

The therapeutic use of non-steroidal anti-inflammatory agents (NSAIDs) is often restricted by the necessity to deliver them at sites very remote from the target organs or tissues; furthermore, the same topical use has to overcome many obstacles, not always completely quantifiable, due to drug diffusion and absorption from the administration form to the site of action.

NSAIDs show another aspect, related to their irritant side effect on the gastro-enteric mucosa, that reduces the possibility of oral administration towards parenteral or local preparations, whose use is, however, limited by the frequent poor water solubility of the active compounds.

Such problems have in many cases been solved by adopting, as a carrier for the drug, polymeric systems which can contain drugs in a physically bound (dissolved, dispersed, included or adsorbed) state or by true chemical linkages along the polymer backbone or as side groups. These latter systems, in which drugs are delivered by chemically or biologically induced cleavage of the covalent bonds, allow one to achieve a more con-

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stant release of the drug for long periods of time. The use of polymers as prodrugs of bioactive agents can thus decrease the required dose, and then the toxicity of the drug, making its solubility and therapeutic efficiency better.

Moreover, if the macromolecular carrier is a natural product, it can be in vivo degraded to small non-toxic and non-immunogenic fragments.

In this context, we attended to systems in which drugs were linked to natural or synthetic polymers (Giammona et al., 1987, 1989a, 1989b). In particular, the potentialities as a drug carrier of a hydrosoluble biodegradable polymer, α,β-poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) (**1**), originally proposed as a plasma expander (Neri et al., 1973) and then investigated in pharmaceutical controlled release systems (Drobnik et al., 1979), have been extensively studied (Giammona et al., 1987).

The hydroxyl moieties present in this polymer permit binding to compounds bearing carboxyl groups, such as several phenylalkanoic NSAIDs, producing a hydrosoluble adduct whose ester bonds can be readily broken in vivo by cell esterases, or also by chemical cleavage when given orally.

To investigate the real effects of this phenomenon upon the pharmacokinetics of some drugs, we decided to prepare and test with respect to analgesic/anti-inflammatory activity, PHEA adducts of two commonly used compounds: naproxen and 4-biphenylacetic acid.

Naproxen (*d*-2-(6-methoxy-2-naphthyl)propionic acid; **2a**) is a non-steroidal anti-inflammatory agent frequently used in the treatment of rheumatic disease (Berry et al., 1978) and which more recently has been applied as an analgesic (Sevelius et al., 1980; Ylikorkola et al., 1980; Huskisson, 1983).

Its therapeutic effects in the treatment of pain and inflammatory diseases seem to be directly related to the serum levels attained (Sevelius et al., 1980; Day et al., 1982).

4-Biphenylacetic acid (BPAA) (**2b**), which represents the active urinary metabolite of Fenbufen, has been shown to possess in in vivo studies an activity comparable to that of the most common anti-inflammatory drugs and even 10-times greater than aspirin (Tolman et al., 1976). In vitro experiments on guinea pig lung homogenates confirmed that BPAA can inhibit prostaglandin biosynthesis (Tolman and Partridge, 1975). Like others NSAIDs, BPAA was shown to be able to reduce inflammatory pain and the action of hyperthermal substances, as well as to attenuate UV light-induced skin erythema in experimental animals (Sloboda and Osterberg, 1976).

Some of us have already studied BPAA with the aim of modifying its aqueous solubility and thus bioavailability, and to reduce the gastrolesive effects. Thus, we obtained both the corresponding lysine salt (data to be published) and a molecular inclusion form with β-cyclodextrin (Puglisi et al., 1989) whose influence on drug release has been demonstrated in vitro (Castelli et al., 1989) and in vivo (data to be published).

Materials and Methods

Apparatus

IR spectra were recorded using a Perkin-Elmer 1720 Fourier transform spectrophotometer, in potassium bromide discs.

UV spectra were taken on a Perkin-Elmer 330 instrument equipped with a 3600 data station.

Elemental analyses (C, H, N) were carried out on a Carlo Erba model 1106 analyzer; compounds were quantitatively dried before analysis on P_2O_5 under reduced pressure (10^{-3} mmHg) at room temperature for 48 h.

Measurements of viscosity were carried out on an Ubbelhode Viscometer (outflow time, 100–200 s; $T = 25\,^{\circ}C$).

TLC (silica gel $60F_{254}$, Merck, F.R.G.: ethyl acetate/cyclohexane, 80 : 20, v/v; chloroform/methanol/ethyl acetate, 60 : 20 : 20, v/v) was used to check the purity of compounds.

Chemicals

Naproxen was obtained from Sigma Chemical Co., St. Louis (U.S.A.); 4-biphenylacetic acid was from Janssen (Belgium) and was recrystallized from ethanol.

DL-Aspartic acid, ethanolamine and 1,1′-carbonyldiimidazole (CDI) were purchased from

Fluka (Switzerland); *N*,*N*-dimethylformamide (Hoechst) was dried on P_2O_5 and successively distilled under reduced pressure before use.

α,β-Poly(*N*-hydroxyethyl)-DL-aspartamide was prepared via polysuccinimide by polycondensation of DL-aspartic acid in the presence of H_3PO_4 at 180 °C, followed by reaction with ethanolamine in DMF solution (Neri et al., 1973); PHEA was isolated by precipitation in chloroform and collected, washed several times with $CHCl_3$ and dried. The crude product was dissolved in DMF and poured into acetone. The mother liquor, diluted with water, was neutral to pH paper. Aqueous PHEA solution was then dialyzed for 3 days against several changes of distilled water using Visking dialysis tubing (18/32 inch) with a molecular weight cutoff of 12000–14000. IR bands of PHEA were in agreement with literature data (Giammona et al., 1987).

Determination of intrinsic viscosities

Intrinsic viscosities $[\eta]$ were determined by measuring the reduced viscosity in the range 2–10 mg/ml, and extrapolation of the concentrations to zero. Polysuccinimide was dissolved in 0.1 M LiCl in dimethylformamide (DMF) [M = 30 900 according to the Mark–Houwink equation: $[\eta] = 1.32 \times 10^{-2}\ M^{0.76} = 33.7$ ml/g (Vlasák et al., 1979)], and PHEA in water, [$M = 27100$ according to the Mark–Houwink equation: $[\eta] = 2.32 \times 10^{-3}\ M^{0.87} = 16.7$ ml/g (Antoni et al., 1974)].

*PHEA-naproxen adduct (**3a**)*

A solution of 0.5 g of CDI (3.08×10^{-3} mol) in 2.5 ml of anhydrous DMF was added dropwise, at 0 °C, to a solution of 0.5 g (2.17×10^{-3} mol) of naproxen dissolved in 3.5 ml of dry DMF. To the mixture, kept at 0 °C for 30 min, a solution of PHEA (1 g, 3.69×10^{-5} mol) in dry DMF (12 ml) was added dropwise.

The reaction mixture was maintained for 8 min at 0 °C, for 1 h at 9 °C, and then set aside at room temperature for 3 days with occasional shaking. Finally, it was poured into 200 ml of butanol in which **3a** is not soluble. The resulting solid, filtered off and washed several times with acetone, was then solubilized in 30 ml of water and purified by exhaustive dialysis against deionized water using Visking dialysis tubing (18/32 inch) with a molecular weight cutoff of 12000–14000.

The purified aqueous solution was subjected to evaporation at 40 °C under reduced pressure and then dried.

The pure adduct was obtained in a yield of 96%, based on the starting material (PHEA). The IR spectrum of **3a** showed bands at 3300 (OH, broad), 1730 (ester C=O), 1650 (amide C=O) and 1540 cm^{-1} (amide II band). The content of active agent in the adduct was determined by both UV spectroscopy and hydrolysis at pH 10 (see Results).

Analysis – Calcd. for $C_{8.24}H_{11.24}N_2O_{3.32}$ (related to 16% of substitution): C, 51.52; H, 6.21; N, 14.59. Found: C, 51.18; H, 6.33; N, 14.83.

*PHEA-BPAA adduct (**3b**)*

The adduct was prepared following the same procedure as that for **3a**, starting from 0.46 g of BPAA (2.17×10^{-3} mol) in 3.5 ml of dry DMF, 0.5 g (3.08×10^{-3} mol) of CDI in 2.5 ml of dry DMF, and 1 g (3.69×10^{-5} mol) of PHEA in 12 ml of dry DMF. The crude product was obtained by pouring the reaction mixture into butanol (200 ml) in which it is insoluble. The filtered solid was washed with acetone, dissolved in 30 ml of water and purified by dialysis.

Yield: 97% based on PHEA. The IR spectrum presented bands at 3300 (OH, broad), 1730 (ester C=O), 1650 and 1540 cm^{-1} (amide I and II, respectively). The content of active agent was determined by UV spectrophotometry and hydrolysis at pH 10 (see Results).

Analysis – Calcd. for $C_{8.1}H_{11.5}N_2O_{3.15}$ (related to 15% of substitution): C, 51.94; H, 6.18; N, 14.96. Found: C, 52.13; H, 6.27; N, 15.22.

*Hydrolysis of the polymeric prodrugs **3a** and **3b** at pH 10*

Ten mg of adduct **3a** or **3b** were dissolved in 10 ml of pH 10 buffer solution (H_3BO_3/KCl/0.1 N NaOH).

The reaction mixture was maintained at 70 °C for 30 h, after which it was neutralized with 1 N HCl and the solvent evaporated off in vacuo. The resulting crude product was treated with 20 ml of acetone and refluxed for 3 h.

TABLE 1

Release of naproxen from **3a** adduct at pH 1.1

Time (h)	Conc. [a]
2	0.768
3	1.034
5	1.395
7	1.596
9	1.976
11	2.307
14	3.856
16	4.811
18	5.378
20	5.976

[a] % w/w of naproxen.

The suspension was then filtered and the solid material separated from the organic mother liquor. The solid obtained, dissolved in water, shows no UV absorption at the characteristic wavelengths of the polymeric prodrug or free drug. Thereafter, the acetone mother liquor was evaporated under reduced pressure and the solid material obtained was solubilized in a suitable volume of ethanol in order to determine the amount of drug released by hydrolysis of the adducts **3a** or **3b**. The amount of active agent was determined by UV spectrophotometry at the absorption maximum of the free drug. ($E^{1\%}_{271.5} = 223.21$, in ethanol, for naproxen and $E^{1\%}_{254} = 938.48$, in ethanol, for BPAA).

The load of naproxen in **3a** was 16.88%, the amount of BPAA in **3b** being 15.16%.

Hydrolysis of adducts at pH 1.1

Ten aliquots of 10 mg of **3a** or **3b** were dissolved in pH 1.1 buffer solution (HCl, NaCl and glycine) (10 ml), maintained at $37 \pm 0.1°C$ and sampled at suitable different intervals.

Each sample, after neutralization with 1 N NaOH, was treated as described above for hydrolysis at pH 10 and the drug concentration measured by UV spectrophotometry at the absorption maximum, in ethanol. Tables 1 and 2 report the release rate of drugs from the PHEA adducts **3a** and **3b**, respectively.

TABLE 2

Release of BPAA from **3b** adduct at pH 1.1

Time (h)	Conc. [a]
2	0.633
3	1.215
5	1.437
7	1.875
9	2.213
11	2.403
14	3.748
16	5.011
18	5.487
20	6.213

[a] % w/w of BPAA.

Pharmacology

Prodrugs **3a** and **3b** described in this paper were screened for their analgesic and anti-inflammatory activity. Male Swiss mice (23–26 g) and male Sprague–Dawley rats (150–170 g) were used. The adducts **3a** and **3b** were carried by 0.5% methylcellulose in water and administered orally. The animals were starved for about 15 h before administration. The properties of test adducts **3a** and **3b** were compared with those of 4-biphenylacetic acid and naproxen. Statistical analysis was performed by using Student's *t*-test vs. controls. The level of significance was set at $P \leqslant 0.05$.

Analgesic activity (phenylquinone writhing test) (Berkowitz et al., 1977)

Groups of 6 mice were injected i.p. with 0.25 ml/mouse of a 0.02% hydroalcoholic solution of phenylquinone 4 h after oral administration of the test drugs. The writhing response frequency was counted in each animal for 5 min (between the 5th and 10th minute) after injection of the irritant. The analgesic effect was expressed as percentage of protection in comparison with controls.

Anti-inflammatory activity (carrageenin-induced oedema) (Winter et al., 1962)

Groups of 6 rats were used. Thirty minutes after oral administration of the test compound, 0.1 ml of a 0.1% carrageenin solution was injected into the sub-plantar tissue of the right paw and the volume was measured by a mercury plethysmometer. The increase in volume of the paw 4 h after the injection of carrageenin was adopted as a measure of the oedema. Swelling in

treated animals was calculated as percentage of inhibition in comparison with controls.

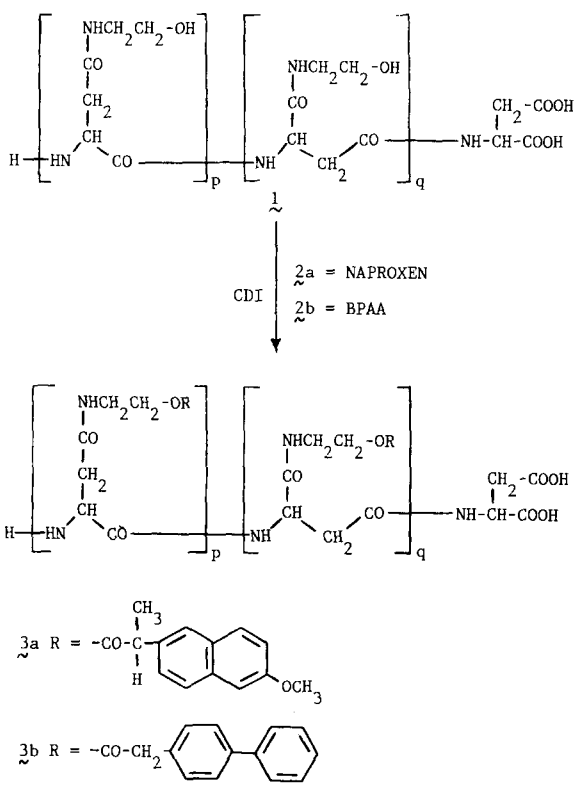
Results and Discussion

Naproxen and BPAA can be linked to PHEA as a polymeric carrier by means of CDI (Ferruti and Vaccaroni, 1975; Ferruti, 1985).

CDI reacts with carboxylic acids affording activated *N*-acyl derivatives which undergo reaction with hydroxylated compounds such as PHEA under mild conditions. The synthesis of the polymeric prodrugs **3a** and **3b** is reported in Scheme 1.

The covalent conjugate drug-polymer bond was demonstrated by UV, IR, and elemental analysis.

The absence of the free drugs in adducts **3a** and **3b**, purified by washing with acetone (in which only the free drugs are soluble) and dialysis (see Materials and Methods), has been indicated by TLC; UV analysis of the adducts presented absorption bands within the range 230–350 nm, in which the polymeric carrier did not absorb. IR spectra of the adducts showed the typical band of the ester C=O group at 1730 cm^{-1} which was not present in the spectrum of PHEA alone.



Scheme 1.

The content of active agents in adducts **3a** and **3b** was determined by hydrolysis, UV spectroscopy and elemental analysis. The amounts of naproxen in **3a** and BPAA in **3b** were determined to be about 16 and 15%, respectively.

UV measurements were made in ethanol/water (70 : 30, w/w) at the same absorption maximum for naproxen [$E^{1\%}_{272} = 242.66$] and PHEA-naproxen adduct **3a** [$E^{1\%}_{272} = 39.34$], and for BPAA [$E^{1\%}_{254} = 944.38$] and PHEA-BPAA adduct **3b** [$E^{1\%}_{254} = 144.67$].

Linking drugs to the carrier did not cause any shift in the absorption maximum (Figs. 1 and 2). PHEA did not interfere with the absorption of the active components.

The ethanol/water (70 : 30, w/w) mixture employed as solvent for UV determinations was chosen in order to permit the solubilization of both the free drugs and macromolecular prodrugs.

The amounts of drugs in PHEA-NSAIDs adducts have also been evaluated by carrying out hydrolysis in a pH 10 buffer solution and at $70 \pm 0.1°$ C for 30 h, by which time hydrolysis had reached completion. Reliable data thus obtained agree with the UV analysis, as reported in Materials and Methods.

Moreover, since the carboxyl group of drugs **2a** and **2b** is essential to their therapeutic action, a polymeric prodrug of prolonged action must be designed in a form from which the active agent can be cleaved in its original state. Therefore, the hydrolysis of adducts **3a** and **3b** in simulated gastric juice (buffer pH 1.1 solution) at $37 \pm 0.1°$ C was studied. Under this set of conditions, the results obtained demonstrate the formation of the free drugs from the macromolecular support, such as in the pH 10 hydrolysis, and a delayed release of the drugs.

Analgesic and anti-inflammatory activity of adducts **3a** and **3b** was studied in comparison to equivalent amounts of the free drugs.

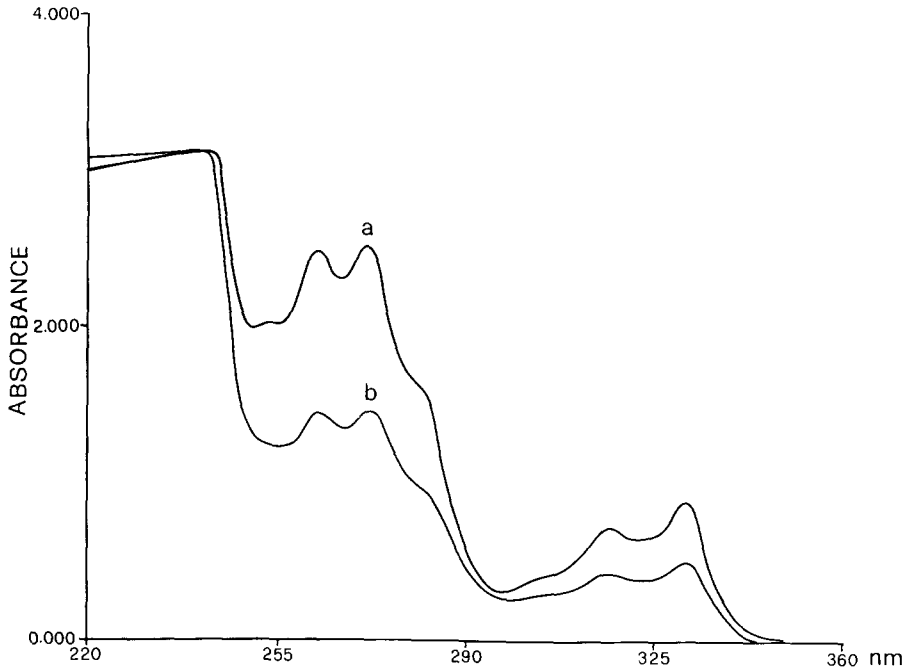


Fig. 1. UV absorption of naproxen (a) and PHEA-naproxen adduct (b) in ethanol/water (70 : 30, w/w).

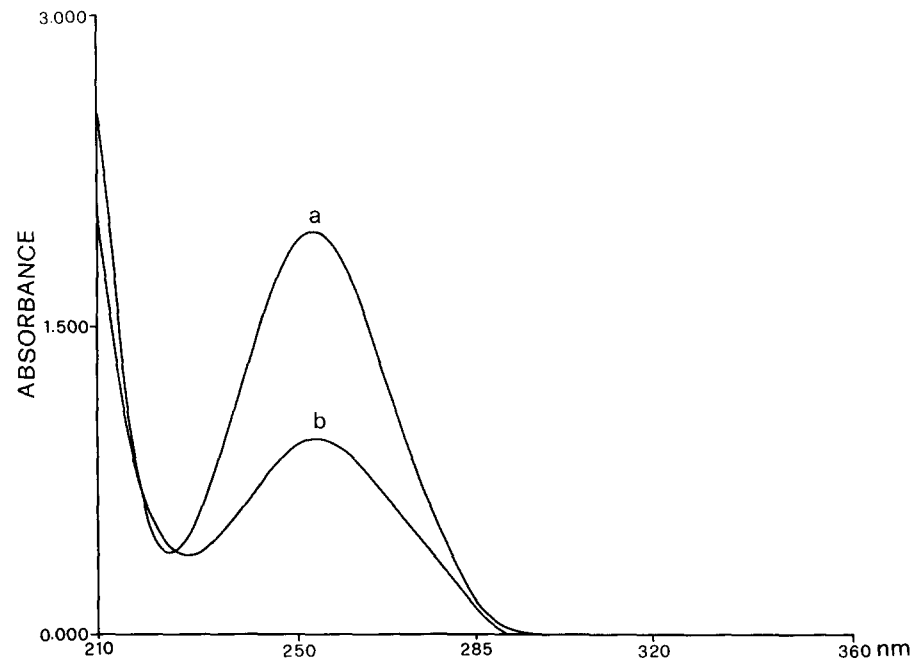


Fig. 2. UV absorption of BPAA (a) and PHEA-BPAA adduct (b) in ethanol/water (70 : 30, w/w).

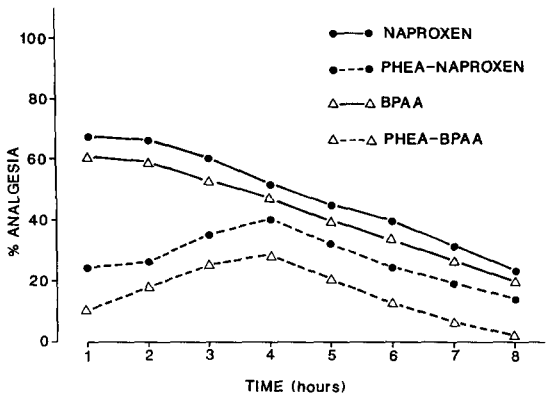


Fig. 3. Time course of naproxen (●———●), PHEA-naproxen adduct (●- - - - -●), BPAA (△———△), and PHEA-BPAA adduct (△- - - - -△) on phenylquinone writhing test (10 mg/kg per os).

The writhing test for analgesic activity was performed with a dose of adduct corresponding to 10 mg/kg of naproxen and BPAA, respectively (Fig. 3). Anti-inflammatory activity (carrageenin paw oedema) was tested on amounts of prodrugs containing 5 or 10 mg/kg and 40 or 80 mg/kg of the two drugs, respectively (see Fig. 4).

In all of the tests, the macromolecular adducts revealed the maintenance of biological activity. In both analgesic and anti-inflammatory activity, they showed maximum response at about 4 h subsequent to administration, whereas the free drugs attained their greatest level of activity after 1 h, thereafter decreasing continuously.

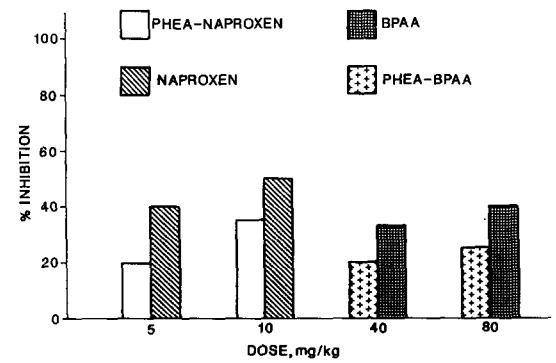


Fig. 4. Anti-inflammatory activity (carrageenin rat paw oedema test) of naproxen, PHEA-naproxen adduct, BPAA, and PHEA-BPAA adduct at the 4th hour after administration.

Throughout the tests, the activity of the free drugs was always higher than equivalent amounts of carrier-bound drugs. These results, often found in the literature (Zaffaroni and Bonsen, 1978), may be ascribed to the hydrolysis rate of the adducts, as a consequence of the chemical modification to which the drug molecule was subjected.

The use of a polymeric carrier, however, allows one to achieve the sustained release of drugs at therapeutic concentrations, probably as a result of the slower metabolism of the drugs; moreover, it can produce a vehicle for the active compounds that is readily water-soluble, improving, at the same time, their pharmacological efficiency.

Acknowledgement

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