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Development of noncytotoxic PLGA nanoparticles to improve the effect of a new inhibitor of p53–MDM2 interaction

Ana M. Paiva[a,b], Rita A. Pinto[c], Maribel Teixeira[a,d,*], Carlos M. Barbosa[a,e], Raquel T. Lima[a,c], M. Helena Vasconcelos[a,c,f], Emília Sousa[a,b,**], Madalena Pinto[a,b]

[a] Centro de Química Medicinal – Universidade do Porto (CEQUIMED-UP), Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n° 228, 4050-313 Porto, Portugal
[b] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n° 228, 4050-313 Porto, Portugal
[c] Cancer Drug Resistance Group, IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Rua Dr. Roberto Frias, S/N, 4200-465 Porto, Portugal
[d] Centro de Investigação em Ciências da Saúde (CICS), Instituto Superior de Ciências da Saúde – Norte, CESPU, Rua Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[e] Laboratório de Tecnologia Farmacêutica, Departamento de Ciências do Medicamento, Faculdade de Farmácia, Universidade do Porto, Portugal
[f] Laboratório de Microbiologia, Departmento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal

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ABSTRACT

One possible approach to overcome solubility complications and enhance the biological activity of drugs is their incorporation into drug delivery systems. Within this scope, several nanosphere and nanocapsule formulations of a new inhibitor of p53–MDM2 interaction (xanthone **1**) were developed and their physicochemical properties analyzed. Through the investigation of the effect of several empty nanoparticles on the growth of MCF-7 cells, it was possible to observe that four out of five formulations were cytotoxic and that some correlations between the toxic potential of these polymeric nanoparticles and their properties/composition could be extrapolated. One empty formulation of nanocapsules developed by emulsification/solvent evaporation and containing PLGA, PVA and Mygliol® 812 was found to be non-cytotoxic to this cell line. The corresponding compound **1**-loaded nanocapsules showed an incorporation efficiency of 77% and revealed to be more potent than the free drug against cell growth inhibition, which may be related to the enhancement in its intracellular delivery. In an integrative study, the intracellular uptake of nanocapsules was confirmed using fluorescent 6-coumarin and well as compound **1** release from nanocapsules. Overall, it was possible to enhance the effect of the hit inhibitor of p53–MDM2 interaction through the development of suitable noncytotoxic polymeric nanoparticles.

1. Introduction

The pharmacological relevance of xanthone derivatives has led the scientific community to isolate or synthesize xanthonic compounds in the search for novel drug candidates (Azevedo et al., 2012; Pinto et al., 2005). In the past few years, a large number of naturally-occurring and synthetic prenylated xanthones has been reported, particularly some with antitumor activity (Azevedo et al., 2012; Pinto and Castanheiro, 2009). Pre-clinical studies of natural prenylated xanthones have already suggested the extremely low oral bioavailability for the most investigated prenylxanthone, α-mangostin (Fig. 1) (Chitchumroonchokchai et al., 2013; Li et al., 2011). Recently, a dihydropyranoxanthone, synthetized by some of us, 3,4-dihydro-12-hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-b]xanthen-6-one (**1**, Fig. 1), presented significant antiproliferative and apoptotic inducing effects (Paiva et al., 2012; Palmeira et al., 2010) in human tumor cell lines. Both α-mangostin (Leão et al., 2013a) and compound **1** (Leão et al., 2013b) were shown to be promising inhibitors of p53–MDM2 interaction, with compound **1** showing the highest inhibitory activity in a yeast target-based assay, mimicking the activity of known p53 activators. In addition, compound **1** was shown to inhibit P-glycoprotein in leukemia cells and presented an apparently high permeability coefficient across the human colon cancer cell line (Caco-2) (Sousa et al., 2012).

* Corresponding author at: CICS, Instituto Superior de Ciências da Saúde – Norte, CESPU, Rua Central de Gandra 1317, 4585-116 Gandra PRD, Portugal. Tel.: +351 220428689; fax: +351 226093390.
** Corresponding author at: CEQUIMED-UP, Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n° 228, 4050-313 Porto, Portugal. Tel.: +351 220428689; fax: +351 226093390.
E-mail addresses: maribel.teixeira@iscsn.cespu.pt (M. Teixeira), esousa@ff.up.pt (E. Sousa).

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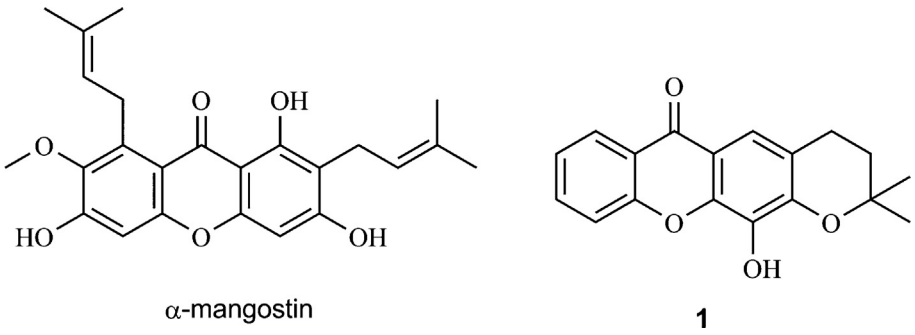

Fig. 1. Representative prenylated xanthones inhibitors of p53–MDM2 interaction: α-mangostin and the target molecule of this study, 3,4-dihydro-12-hydroxy-2,2-dimethyl-2*H*,6*H*-pyrano[3,2-b]xanthen-6-one (**1**).

As for the majority of promising new compounds, the success of compound **1** and other xanthone derivatives may be compromised by their poor solubility. In general, apart from the difficulty associated with the administration of water-insoluble drug substances, this property is often linked with poor bioavailability. One possible approach to overcome poor physiochemical properties and enhance the bioavailability of drugs is to associate the drug with a pharmaceutical carrier – a drug delivery system (DDS) – which may enhance drug pharmacokinetics and cellular penetration (Chen et al., 2011).

Aliphatic poly(esters) like poly(lactide), poly(glycolide) and specially poly(D,L-lactide-co-glycolide) (PLGA) have been the most extensively investigated polymers for drug delivery, due to their excellent biocompatibility and biodegradability. Drugs entrapped in this type of polyester polymer matrix are released at a sustained rate, through diffusion of the drug in the polymer matrix and by degradation of the polymer matrix (Jong and Borm, 2008). Nanoparticles are submicron sized colloidal polymeric systems and according with the methods used for their preparation nanospheres or nanocapsules can be obtained. Nanospheres are matrix-type systems in which a drug is dispersed throughout the particles, whereas nanocapsules are vesicular systems in which a drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane (Reis et al., 2006). This work is an integrated study that includes physicochemical characterization and biological analysis of compound **1**-loaded polymeric nanoparticles which demonstrates uptake, and effect on the growth of a human breast adenocarcinoma cell line (MCF-7). In the present work, several polymeric nanosystems, nanocapsules and nanospheres, incorporating compound **1** were developed by different techniques: solvent displacement (SD), emulsification/solvent diffusion (ESD), and emulsification/solvent evaporation (ESE), and some formulation factors were studied in order to obtain nanoparticles with favorable technological characteristics. The cytotoxicity of both empty and loaded nanoparticle formulations was accessed in the MCF-7 (human breast adenocarcinoma) cell line, which was critical for the selection of the most suitable formulation. Furthermore, the intracellular uptake of nanocapsules containing a fluorescent probe (6-coumarin) was also investigated in the same cell line.

2. Materials and methods

2.1. Materials

3,4-Dihydro-12-hydroxy-2,2-dimethyl-2*H*,6*H*pyrano[3,2-*b*]xanthen-6-one (**1**) was obtained by a previously described method (Palmeira et al., 2010) and showed a purity of 98.5% by HPLC-DAD. PLGA 50:50 (M_W: 50,000–75,000 Da), Pluronic® F-68, glucose, polyvinyl alcohol (PVA), 6-coumarin, Tween® 80 and Span® 80 were purchased from Sigma–Aldrich Química (Sintra, Portugal) and Mygliol® 812 was purchased from Acofarma (Coimbra, Portugal). HPLC grade reagents methanol, acetonitrile and acetic acid were obtained from Carlo Erba Reagents, (Val de Reuil, Italy) and ultra-purified water was produced by a Millipore Milli-Q system (Simplicity® UV Ultrapure Water System, Millipore Corporation, Billerica, USA). All the other reagents and solvents were of analytical or HPLC grade.

2.2. Apparatus and chromatographic conditions

The HPLC analysis was performed in a Finnigan Surveyor – Autosampler Plus and LC Pump Plus, Thermo Electron Corporation (Ohio, USA), equipped with a diode array detector TSP UV6000LP, and using a C-18 column (5 μm, 250 mm × 4.6 mm I.D.) from Macherey-Nagel (Düren, Germany). The injected volume was 20 μl and the eluent was monitored at 254 nm. Xcalibur® 2.0 SUR 1 software, Thermo Electron Corporation (Ohio, USA) managed chromatographic data.

2.3. Preparation of nanospheres

Nanospheres containing compound **1** were prepared by SD with some modifications to the previously described methods (Fessi et al., 1989; Zili et al., 2005) (Table 1, formulations I–III). Briefly, an organic solution of **1**, polymer, and containing or not a lipophilic surfactant was poured, under magnetic stirring into 10 ml of aqueous solution of a hydrophilic surfactant (Pluronic® F-68 or Tween® 80). After 5 min of stirring, nanosphere dispersions were concentrated to 5 ml under reduced pressure. Separation of non-incorporated compound was performed first by filtration (membrane with a porosity of 0.45 μm), and then by centrifugation at 1830 rpm for 30 min (Sigma 1–14, Osterode am Harz, Germany) after solubilization of a certain amount of glucose for achieving a 5% (w/v) concentration, in order to avoid aggregation of the particles during the centrifugation step. The supernatant was discarded and the pellet containing the nanospheres was redispersed in water to complete the initial volume (5 ml).

The development of nanospheres containing compound **1** prepared by ESD was based on a previously described procedure (Quintanar-Guerrero et al., 1996) with some modifications (Table 1, formulations IV–V). Briefly, the organic phase containing the polymer and the surfactant was poured into 10 ml of the aqueous phase, while mixing with an high speed homogenizer (20,000 rpm for 5 min, IKA-T18 basic, Ultra Turrax®, Germany) or by sonication (130 W, 90 s, VibraCell model-75186, Sonics, USA), to form an oil in water nanoemulsion, followed by evaporation under reduced pressure until the final volume of 5 ml was reached. A certain amount of glucose for achieving a 10% (w/v) concentration was solubilized and the separation of non-incorporated compound was performed first by filtration (membrane with a porosity of 0.45 μM) and then