International Journal of Pharmaceutics 491 (2015) 323–334

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Phthalimido–ferrocidiphenol cyclodextrin complexes: Characterization and anticancer activity

Feten Najlaoui[a,f,g], Pascal Pigeon[b,c], Zaineb Abdelkafi[a], Sebastien Leclerc[d], Pierrick Durand[e], Mohamed El Ayeb[a], Naziha Marrakchi[a], Ali Rhouma[f], Gérard Jaouen[b,c], Stéphane Gibaud[g,*]

[a] Institut Pasteur de Tunis, Laboratoire des Venins et Biomolécules Thérapeutiques LR11IPT08, 13, Place Pasteur, 1002 Tunis, Tunisia
[b] Chimie ParisTech, 11 rue Pierre et Marie Curie, Paris F-75231 Paris Cedex 05, France
[c] Sorbonne Universités, UPMC Université Paris 6, Institut Parisien de Chimie Moléculaire (IPCM), UMR 8232, 4 Place Jussieu, 75252 Paris Cedex 05, France
[d] Université de Lorraine, LEMTA, UMR 7563, Faculté des Sciences et Technologies, Boulevard des Aiguillettes, Vandœuvre-lès-Nancy F-54500, France
[e] Université de Lorraine/CNRS, CRM2, UMR 7036, Faculté des Sciences et Technologies, Boulevard des Aiguillettes, Vandœuvre-lès-Nancy F-54500, France
[f] Olive Tree Institute, Research Unit of Plant Protection and Environment, Mahrajene City BP 208, 1082 Tunis, Tunisia
[g] Université de Lorraine, EA 3452/CITHEFOR, 5 rue Albert Lebrun (Faculté de Pharmacie), F-54000 Nancy, France

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 22 June 2015
Accepted 23 June 2015
Available online 29 June 2015

Keywords:
Ferrocene
Organometallic
Anticancer drug
Glioma
Cyclodextrin

ABSTRACT

Several ferrocenyl analogues of tamoxifen have already showed strong antiproliferative activity in experimental glioma models. Nevertheless, these compounds are very poorly soluble in water and an adapted formulation is needed.

In this work, we have tailored and optimized methylated cyclodextrin soluble complexes of phthalimido–ferrocidiphenol for the first time. The complexes were characterized, and the optimized formulation was tested for *in vitro* efficacy and cell proliferation assays on U87, human glioblastoma cancer cells.

Molecular modeling can provide accurate information about the inclusion process. The inclusion of all the moieties at the same time (*i.e.*, ferrocene, phthalimidylpropyl, 2 phenols) is not possible due to the steric hindrance of the 1:4 system. The 1:3 systems are possible but do not seem very relevant. However, various 1:2 and 1:1 complexes are mostly present in aqueous solutions.

Some experiments have confirmed our hypothesis. First, interactions between the phenol, phthalimidylpropyl and ferrocenyl groups have been observed in our NMR experiments. Second, the inclusion of phthalimidylpropyl was detected by UV–vis spectrophotometry with an apparent 1:1 interaction, which was observed through the Benesi–Hildebrand method.

The complex is readily soluble in water and keeps its pharmacological activity against U87 tumor cells ($IC_{50} = 0.028 \pm 0.007$ μM *vs.* 0.018 ± 0.003 μM for PhtFerr).

1. Introduction

Ferrocene has gained tremendous attention in the scientific and technical community because of its captivating chemistry (Rosenblum, 1965; Togni and Hayashi, 1994). Different strategies have been adopted to develop ferrocene derivatives and to explore their wide-ranging scientific applications.

Ferrocenyl compounds have gained much popularity in biological applications because of their stability in aqueous and aerobic media, access to a great variety of derivatives, and their promising electrochemical properties: the toxic agent in the cell can eventually transform into an oxidized and reactive molecule. Thus, we have proven that molecules bearing the [ferrocenyl-ene-phenol] motif can be oxidized into a quinone methide (Buriez et al., 2008; Messina et al., 2012; Wang et al., 2015) able to react with a SH moiety, as in some proteins, or on the selenocysteine residue of thioredoxin reductases (Citta et al., 2014). The formation of this molecule is due to the redox properties of ferrocene (Hillard et al., 2010; Swarts et al., 1994). More specifically, recent studies have shown a high activity of ferrocene derivatives *in vitro* and *in vivo* against a variety of fungal and bacterial infections (Biot et al., 2000; Zhang, 2008), *e.g.*, malaria (Biot et al., 1997; Fouda et al., 2007; Itoh et al., 2000), HIV (Kondapi et al., 2006) and cancer (Hillard et al.,

* Corresponding author. Fax: +33 3 83 68 23 01.
E-mail address: stephane.gibaud@univ-lorraine.fr (S. Gibaud).

http://dx.doi.org/10.1016/j.ijpharm.2015.06.043
0378-5173/

2010; Swarts et al., 1994). After these results, the medicinal use of ferrocene became plausible.

Consequently, a variety of ferrocenyl compounds are being synthesized and tested for their anticancer properties. Among all ferrocene derivatives, ferrocifens (Jaouen and Top, 2013) have encouraging effects against breast cancer (Hillard et al., 2010; Nguyen et al., 2007). Recently, the non-steroidal selective estrogen receptor modulator (SERM), tamoxifen, has been commonly prescribed for patients diagnosed with estrogen-receptor-positive (or ER+) breast cancer (Craig Jordan, 1992). The first coupling of ferrocene to the active metabolite of tamoxifen was done by G. Jaouen and his co-workers by replacing a phenyl group of 4-hydroxytamoxifen with ferrocene (Ornelas, 2011). These hydroxyferrocifens were intended to combine the antiestrogenic properties of tamoxifen while targeting the ferrocenyl moiety to the receptor and thus to DNA for a possible gain in therapeutic benefits (Top et al., 1996, 2003).

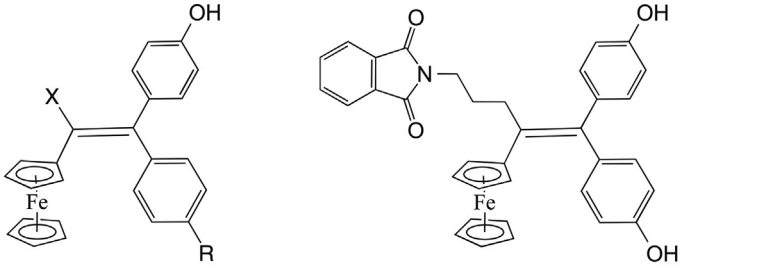
In addition to the effect of the quinone methide, it was also suggested that DNA damage due to hydroxyl radical production causes the toxicity of the ferrocenium cation (Tamura and Miwa, 1997). To bring ferrocene in close proximity to DNA, the ferrocenyl group can be attached to molecules that bind DNA, which might enhance the probability of DNA damage and cell apoptosis (Ornelas, 2011).

The molecules bearing the [ferrocenyl-ene-phenol] motif, which are needed to form the reactive quinone methides *in vivo*, are called the ferrociphenol series. The hydroxyferrocifen series bearing this motif (and also a dimethylaminoalkyl chain inherited from the tamoxifen) belong to this larger family. The replacement of this chain by a second phenol group led to the ferrocidiphenol family, a sub-series of the ferrociphenol series (Fig. 1), without many change in the activity, and with the advantage of having a smaller size and avoiding formation of a Z/E isomers mixture. Thus, a series of ferrociphenols [ferrocifens (Top et al., 2003), ferrocidiphenol (Vessieres et al., 2005), and cyclic ferrocidiphenols (Plazuk et al., 2009)] that was evaluated for their antiproliferative activity against breast cancer displayed low IC_{50} values (between 0.09 and 13 μM) (Hillard et al., 2010). Additionally, several ferrocenyl analogues of tamoxifen have already showed a strong antiproliferative activity on experimental glioma models (Laine et al., 2013; Roger et al., 2012). This property is a matter of interest, as glioblastoma, which is the most common malignant glioma, has only been associated to date with a median survival of 12–15 months. In fact, the European Organization for Research and Treatment of Cancer (EORTC) has published a study on the concomitant use of radiation therapy and adjuvant temozolomide, which slightly improved survival (overall survival: 9.8% after 5 years). This treatment is now adopted as the new standard treatment, but it is still not satisfactory (Stupp et al., 2009). Temozolomide is an oral drug that is rapidly metabolized into methyltriazeno-imidazole-carboxamide (MTIC), a DNA-methylating drug. A DNA repair enzyme, methyl-guanine methyltransferase (MGMT), can remove the methyl group and overcome the modification of cells that lack MGMT, which have been shown to have a higher sensitivity to temozolomide. Intrinsic or acquired resistance often defines the poor efficacy of chemotherapy in malignant gliomas.

Having fixed 3 of the 4 substituents of the central alkene double bond of the ferrocidiphenol molecule (ferrocenyl and 2 phenol moieties), the only degree of freedom was to modify the ethyl group, inherited from the tamoxifen. Fortunately, recent work showed us that modifications sometimes permitted a sensible gain in activity [hydroxylation (Pigeon et al., 2014; Richard et al., 2015; Wang et al., 2015) or formation of a ferrocenophane cycle (Plazuk et al., 2009)]. Thus, with this encouraging research on the modification of this ethyl group, a new substituted ferrocidiphenol, a "phthalimido–ferrocidiphenol" (replacement of the ethyl group by a phthalimidylpropyl moiety, PhtFerr, Fig. 1), has emerged and become the most effective agent according to *in vitro* antiproliferative assays (on glioma cells U87). Interestingly, PhtFerr was tested on breast cancer cells (0.20 μM on MDA-MB231; 3-fold better than the original ferrocidiphenol molecule) and on human glioblastoma cancer cells (U87), where it was shown to be very active (unpublished results). To date, its mechanism of action is not fully understood, but an oxygenated polar moiety seems to be needed on the alkyl chain replacing the ethyl group (SAR in progress).

This compound (Fig. 1) is a highly lipophilic molecule (Log $P_{o/w}$: 5.30) that is very poorly soluble in water (solubility <0.001 μg/ml). In the last decade, many solutions have been proposed for the administration of poorly soluble drugs (Bittner and Mountfield, 2002): pH adjustment and co-solvent solutions (Alvarez-Nunez and Yalkowsky, 1999), cyclodextrins (CD) (Buriez et al., 2008; Petrovski et al., 2008), surfactants, mixed micelles (Nguyen et al., 2008; Wei et al., 2010), emulsions (Gibaud and Attivi, 2012), and nanosuspensions (Ben Zirar et al., 2008).

Lipid nanocapsules (LNC) of approximately 50 nm were also used to encapsulate the active ferrocenyl diphenol (ferrocidiphenol or Fc-diOH; Fig. 1) tamoxifen derivative in order to allow for a parenteral administration (Allard et al., 2009; Allard et al., 2008). The Fc-diOH (ferrocidiphenol molecule) was encapsulated in high loading because of the large hydrophobic core in the LNC structure. The loaded LNCs were taken up by 9L-glioma cells, and the cytostatic activity of the Fc-diOH was conserved presenting an IC_{50} value of approximately 0.6 μM.



Ferrociphenol series (and sub-series) :
X = Et, R = O-$(CH_2)_n$NMe$_2$ (ferrocifens series)
R = OH (ferrocidiphenol series)
X = Et, R = OH (ferrocidiphenol molecule)

New derivative of the ferrocidiphenol series : phthalimido-ferrocidiphenol (PhtFerr)

Fig. 1. The chemical structure of phthalimido–ferrocidiphenol: *N*-{4-ferrocenyl-5,5-bis-(4-hydroxyphenyl)-pent-4-enyl}phthalimide—$C_{35}H_{29}FeNO_4$—Molecular weight: 583.45 g/mol.