European Journal of Medicinal Chemistry 117 (2016) 269–282

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Isouronium and *N*-hydroxyguanidinium derivatives as Cell growth inhibitors: A comparative study

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
16 March 2016
Accepted 17 March 2016
Available online 22 March 2016

Keywords:
DNA minor groove binders
Isouronium diaromatic derivatives
Hydroxyguanidinium diaromatic derivatives
Thermal DNA denaturation
Cell growth inhibition
HL-60 cells
Kelly cells
Flow cytometry
Apoptosis

ABSTRACT

Based on the results obtained from a computational study on the suitability of the isouronium and *N*-hydroxyguanidinium cations as hydrogen bond donors/acceptors, the DNA binding of a series of isouronium derivatives was assessed by DNA thermal denaturation experiments and compared to related *N*-hydroxyguanidines. Due to the poor DNA binding observed, the nature of the diaromatic linker was explored by preparing the corresponding amide-linked *bis*-isouronium derivative and measuring its DNA affinity. Next, the inhibitory effects of the isouronium derivatives on cell viability were evaluated in two different cancer cell lines providing IC_{50} values in the range of 36.9–57.4 μM (HL-60, leukemia), and 17.3–33.9 μM (Kelly, neuroblastoma). These values are comparable to those previously found for the *N*-hydroxyguanidine series. Compounds with the –S- linker (**3**, **6**, and **10**) proved to be considerably active in the HL-60 cells and even more active in the Kelly cell line. No correlation was found between DNA minor groove binding and cell growth inhibition; hence, activity may depend on different modes of action. Further studies into the apoptotic potential of these compounds indicated that, besides inhibiting cell viability and proliferation, derivatives **9** and **10**, are significant apoptosis-inducers in both cell lines. Results obtained with HL-60 cells suggest that G_2/M arrest and subsequent apoptosis induced by compound **10** are associated with microtubular depolymerisation, loss of mitochondrial membrane potential and activation of the caspase cascade. Moreover, the effects of compound **10** on cell viability and apoptosis in two non-cancereous cell lines (NIH3T3 and MCF-10A) indicate none or minimal toxicity.

1. Introduction

Compounds that specifically bind with high affinity into the minor groove of DNA have shown potential for the treatment of diseases at a DNA level and, for this reason, minor groove binders (MGBs) have been widely studied as antibacterial, antiprotozoal, antitumor and antiviral agents [1–5]. The structures of these compounds often contain amidine-like functionalities (amidine, guanidine, 2-aminoimidazoline, imidazole). Examples include pentamidine, used for the treatment of African trypanosomiasis [6] or *Pneumocystis carinii* pneumonia and leishmaniasis [7]; berenil, used for animal trypanosomiasis [8]; pyrrole-imidazole polyamides, some of which have exhibited antitumor activity [9]; distamycin-based *bis*-alkenyl polyamides which have potent activity against Gram-positive bacteria by formation of very specific hydrogen bonds (HBs) [10]; or analogues of imidazoline-pyrrole based MGBs that have shown efficacy in human papilloma virus strains *in vitro* [11].

Over the past 15 years we have prepared a large number of symmetric and asymmetric di-aromatic guanidinium and 2-aminoimidazolinium derivatives as DNA MGBs with potential cytotoxic/antiparasitic activity (Fig. 1) [12–17].

Considering the encouraging results obtained with these guanidine-based families, we prepared additional analogues incorporating isouronium and *N*-hydroxyguanidinium functionalities. Our aim was to probe the cationic binding to the DNA minor

Abbreviations List: DNA, deoxyribonucleic acid; AT, adenine-thymine base pair; MGB, minor groove binder; MP2, second order Møller-Plesset calculation; HB, hydrogen bond; CD, circular dichroism; NMR, nuclear magnetic resonance; IR, infrared; HRMS, High resolution mass spectrometry; st, salmon testes; SPR, surface plasmon resonance; $\Delta;T_m$, change in thermal melting temperature; P/D, phosphate/drug; RU, response units; IC_{50}, concentration required for 50% inhibition.

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http://dx.doi.org/10.1016/j.ejmech.2016.03.047
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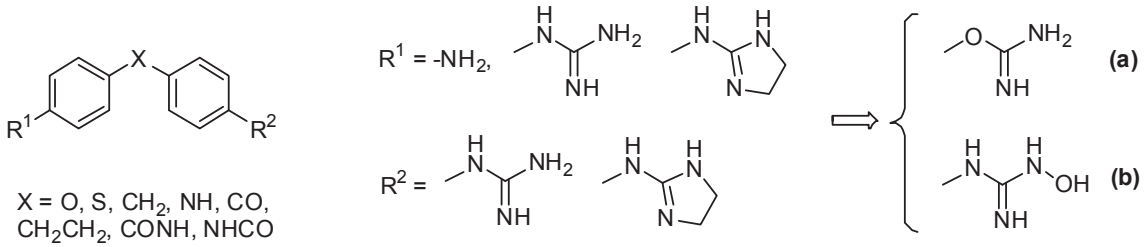

Fig. 1. Generic structure of guanidine-like derivatives developed by Rozas and coworkers [12–17] and structure of the isourea (a) and *N*-hydroxyguanidine (b) functionalities.

groove by introducing an O atom (HB acceptor) connecting the aromatic ring to the amidinium group (isouronium) or by adding an OH functionality (HB donor/acceptor) to the guanidinium cation (*N*-hydroxyguanidinium). We hypothesised that by increasing the number of contacts within the minor groove, improved cytotoxic agents would result. Furthermore, a variety of biophysical measurements including DNA thermal denaturation, SPR, CD, LD and ITC were used to study their binding into the minor groove [6,7].

In particular, we have recently reported the preparation of a series of *bis*-*N*-hydroxyganidine derivatives as potential MGBs, their biophysical properties as well as their cytotoxicity assessment [18]. We found that, even though, somewhat surprisingly, they exhibited poor DNA binding affinity, their ability as growth inhibitors in a number of human cancer cell lines was very promising. Despite the preparation of a series of *bis*-isouronium derivatives being previously reported by our group [19], their biophysical characteristics and, more importantly, their effect on cancer cell growth have not been previously discussed. In this article, we present the discussion of the biophysical and biochemical properties of the *bis*-isouronium derivatives in comparison with the related *bis*-*N*-hydroxyguanidine cationic systems in order to investigate whether the introduction of a HB acceptor in amidine-based cations has an effect on their DNA binding affinity and anticancer activity.

With this comparative aim, we also present a theoretical study of the strength of all the possible HBs formed by the guanidine, isourea and *N*-hydroxyguanidine cations with models of HB donors such as hydrogen fluoride [20] and HB acceptors such as formaldehyde [21] by means of second order Møller-Plesset (MP2 [22]) computations using aqueous solvation (PCM model [23]). Additionally, the results of the study of *bis*-isouronium **1**–**3** derivatives as DNA MGBs in comparison to the previously prepared *bis*-hydroxyguanidiniums **4**–**7** [18] (Fig. 2) are presented. Some *mono*-isouronium derivatives (**8**–**10**, Fig. 2) are included as well in this study as relevant comparisons. Finally, considering that the amide functionality (CONH) is present in many MGBs [4,9,11,24] and that it considerably contributed to improving the binding affinity of our guanidine-like dicationic systems [15], the effect of the CONH linker in the isouronium and *N*-hydroxyguanidinium series was further explored. Thus, the amide-linked *bis*-isouronium was prepared and the DNA binding as well as the growth inhibition in different cell lines was assessed and compared to those of the previously prepared amide-linked guanidine-like dications.

2. Results and discussion

2.1. Theoretical study of isouronium and N-Hydroxyguanidinium cations

An important issue in the biological activity of a potential drug is its pK_a since the basicity of a compound determines the protonation state and hence the absorption of the compound. Accordingly, we have found that experimentally [25–27] and computationally [28] determined, the pK_a values of arylisouroniums (pK_a values of phenylisourea: 8.29 [27] and 4,4′-(*bis*-isourea)diphenylether: 10.4 [26]) and aryl-*N*-hydroxyguanidiniums (pK_a of phenyl-*N*-hydroxyguanidine: 8.9 [28]) are slightly lower than those of arylguanidiniums (pK_a values of phenylguanidine: 10.88 [27] and 4,4′-(*bis*-guanidine)diphenylmethane: 10.5 [25]) or aryl-2-aminoimidazoliniums (pK_a values of phenyl-2-aminoimidazoline: 9.16 [27] and 4,4′-(*bis*-2-aminoimidazoline)diphenylmethane: 9.4 [25]). Thus, there is not a large difference between their pK_a values and consequently all cations would be equally protonated at physiological pH (7.4).

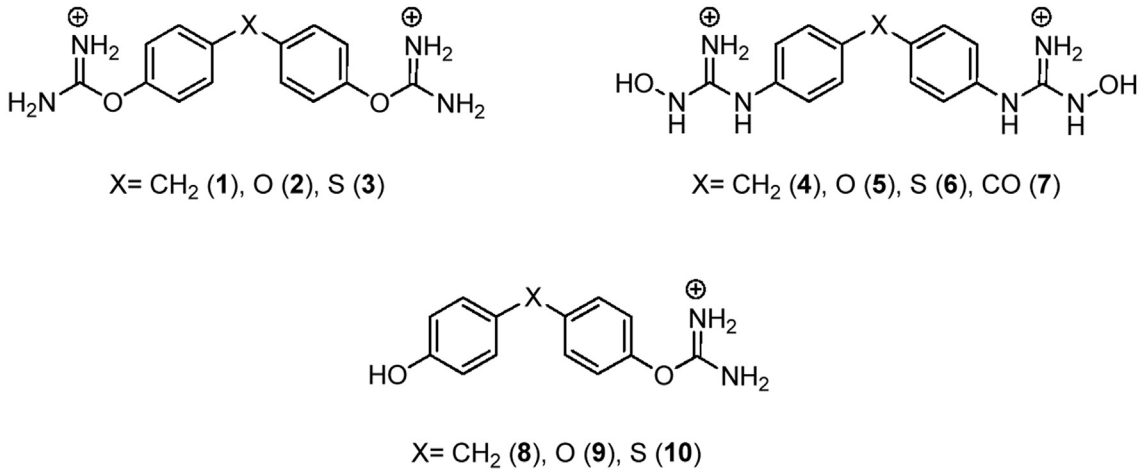

Fig. 2. *Bis*-isouronium (**1**–**3**), *bis*-*N*-hydroxyguanidinium (**4**–**7**) and *mono*-isouronium (**8**–**10**) derivatives here studied.