European Journal of Medicinal Chemistry 107 (2016) 288–294

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Synthesis and evaluation of bis-thiazole derivatives as new anticancer agents

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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form
23 October 2015
Accepted 2 November 2015
Available online 5 November 2015

Keywords:
Bis-thiazole
Anticancer activity
DNA synthesis inhibitory activity
Acetylcholinesterase
Butyrylcholinesterase

ABSTRACT

New bis-thiazole derivatives (**1**–**10**) were synthesized *via* the ring closure of 1,1′-(3,3′-dimethoxybiphenyl-4,4′-diyl)bis(thiourea) with phenacyl bromides and evaluated for their cytotoxic effects on A549 human lung adenocarcinoma, C6 rat glioma, 5RP7 H-*ras* oncogene transformed rat embryonic fibroblast and NIH/3T3 mouse embryonic fibroblast cell lines using MTT assay. DNA synthesis inhibitory effects of these compounds were investigated. Each derivative was also evaluated for its ability to inhibit AChE and BuChE using a modification of Ellman's spectrophotometric method. Among these compounds, 3,3′-dimethoxy-N^4,$N^{4\prime}$-bis(4-(4-bromophenyl)thiazol-2-yl)-[1,1′-biphenyl]-4,4′-diamine (**5**) can be identified as the most promising anticancer agent due to its notable inhibitory effects on A549 and C6 cell lines and low toxicity to NIH/3T3 cell lines. Compound **5** exhibited anticancer activity against A549 and C6 cell lines with IC_{50} values of 37.3 ± 6.8 μg/mL and 11.3 ± 1.2 μg/mL, whereas mitoxantrone showed anticancer activity against A549 and C6 cell lines with IC_{50} values of 15.7 ± 4.0 μg/mL and 11.0 ± 1.7 μg/mL, respectively. Furthermore, compound **5** showed DNA synthesis inhibitory activity against A549 cell line.

1. Introduction

The global burden of cancer is increasing dramatically due to the increased life expectancy. By 2030, the annual number of new cancer diagnoses is projected to be 21 million worldwide, with 17 million patients dying of cancer every year and 75 million people living with cancer diagnoses [1].

Despite the increasing number of anticancer agents developed to date, the lack of selectivity and the acquisition of multiple-drug resistance represent significant impediments to successful cancer treatment and, therefore, extensive efforts have been devoted to the discovery of new potent and selective anticancer agents which destroy tumor cells or at least limit their proliferation [2–7].

Mounting evidence has shown that acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are abnormally expressed in various pathological conditions, such as cancer [8]. In the last few years, AChE has attracted a great deal of interest as a potential therapeutic target for the treatment of cancer due to the involvement of this enzyme in apoptosis and cell adhesion, differentiation, and proliferation [9,10].

In the field of medicinal chemistry, thiazoles are of great interest due to their synthetic feasibility and biological importance. Thiazole ring is present in a large number of biologically active compounds, including natural products and pharmaceutical agents. In the last few decades, the clinical efficacy of tiazofurin and its analogs, and bleomycins (BLMs) has pointed out the importance of thiazole moiety for anticancer drug design. BLMs, glycopeptide antitumor antibiotics produced and isolated from *Streptomyces* sp, have been clinically used to treat several types of cancers, like squamous cell carcinomas, malignant lymphomas and testicular cancers. The bis-thiazole moiety of BLM has been shown to bind to DNA and some researchers have studied the nature of bis-thiazole-DNA interaction extensively [11–23]. Dasatinib, a second-generation tyrosine kinase inhibitor, is an another example of thiazole derivatives used in the treatment of cancer [24].

Encouraged by the afore-mentioned findings and in the continuation of our ongoing research program in the field of design, synthesis and biological evaluation of thiazole derivatives [25–27],

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http://dx.doi.org/10.1016/j.ejmech.2015.11.002
0223-5234/

herein we reported the synthesis of a new series of bis-thiazole derivatives and focused on their potential cytotoxic effects on A549, C6, 5RP7 and NIH/3T3 cell lines. The inhibitory effects of these compounds on DNA synthesis and cholinesterases were also investigated.

2. Results and discussion

The synthesis of bis-thiazole derivatives (**1**–**10**) followed the general pathway outlined in Scheme 1. In the first step, 1,1′-(3,3′-dimethoxybiphenyl-4,4′-diyl)bis(thiourea) (**B**) was synthesized *via* the reaction of 3,3′-dimethoxybiphenyl-4,4′-diamine with potassium thiocyanate in the presence of concentrated hydrochloric acid. Finally, the ring closure reaction of compound **B** with phenacyl bromides afforded 3,3′-dimethoxy-N^4,$N^{4\prime}$-bis(4-phenylthiazol-2-yl)biphenyl-4,4′-diamine derivatives (**1**–**10**). Some properties of the compounds are given in Table 1. The structures of these compounds (**1**–**10**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and elemental analyses.

MTT assay was carried out to evaluate the potential anticancer effects of the compounds on A549 human lung adenocarcinoma, C6 rat glioma and 5RP7 H-*ras* oncogene transformed rat embryonic fibroblast cell lines (Table 2). The cytotoxic effects of the compounds on NIH/3T3 mouse embryonic fibroblast cells were also investigated to determine their selectivity.

Compound **B** showed the lowest anticancer activity against A549 and C6 cell lines. Bis-thiazoles (**1**–**10**) were more effective against both cancer cell lines than bis(thiourea) derivative (**B**). This outcome pointed out the importance of bis-thiazole moiety for anticancer activity against A549 and C6 cell lines.

Generally, the tested compounds exhibited more significant cytotoxic activity against C6 rat glioma cells than A549 human lung adenocarcinoma cells. The most potent cytotoxic agent against A549 cell line was found as compound **5** (IC_{50} = 37.3 ± 6.8 μg/mL) followed by compounds **4** (IC_{50} = 58.3 ± 7.6 μg/mL), **2** (IC_{50} = 82.3 ± 2.5 μg/mL) and **3** (IC_{50} = 98.3 ± 7.6 μg/mL) when compared with mitoxantrone (IC_{50} = 15.7 ± 4.0 μg/mL). Among bis-thiazole derivatives, compound **7** exhibited the lowest anticancer activity against A549 cell line. This result indicated that *p*-bromo substituent enhanced anticancer activity, whilst 3,4-dichloro substituent decreased anticancer activity against A549 cell line.

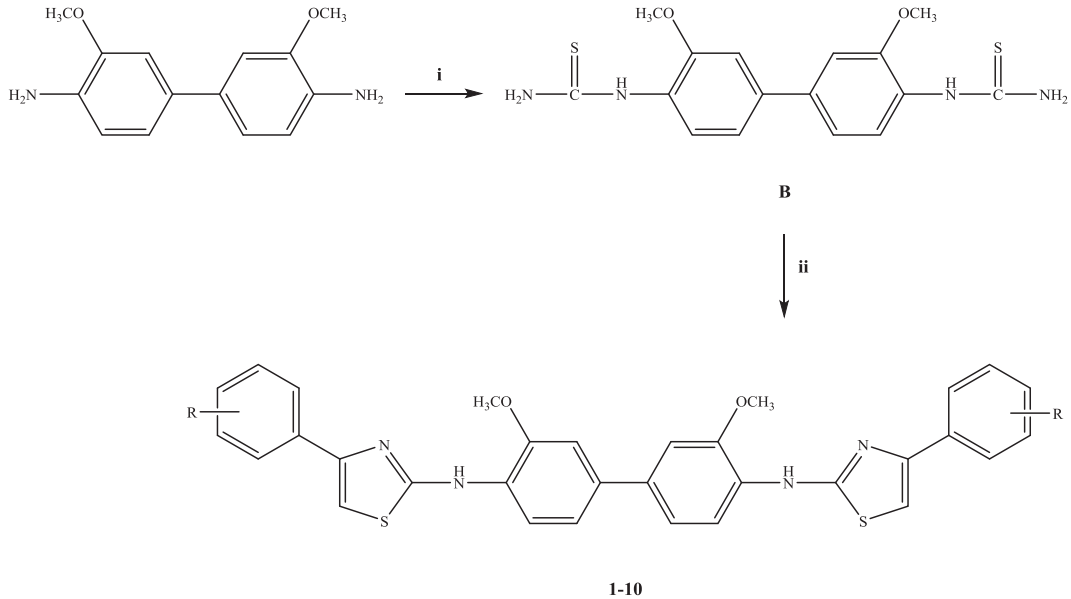
The most effective anticancer agent against C6 cell line was found as compound **5** (IC_{50} = 11.3 ± 1.2 μg/mL) followed by compounds **3** (IC_{50} = 21.0 ± 1.7 μg/mL), **4** (IC_{50} = 28.7 ± 1.2 μg/mL) and **2** (IC_{50} = 33.3 ± 5.8 μg/mL) when compared with mitoxantrone (IC_{50} = 11.0 ± 1.7 μg/mL). Among bis-thiazole derivatives, compound **6** exhibited the lowest anticancer activity against C6 cell line. The MTT assay revealed that *p*-bromo substituent increased anticancer activity, whereas *p*-chloro substituent decreased anticancer activity against C6 cell line.

The most potent cytotoxic agent against 5RP7 cell line was found as compound **2** (IC_{50} = 5.83 ± 1.04 μg/mL) followed by compounds **10** (IC_{50} = 12.33 ± 0.58 μg/mL) and **4** (IC_{50} = 13.33 ± 1.53 μg/mL) when compared with mitoxantrone (IC_{50} = 0.73 ± 0.06 μg/mL). This outcome showed that *p*-nitro, 2,5-dimethoxy and *p*-methoxy substituents increased cytotoxic activity against 5RP7 cell line. On the other hand, compound **5** exhibited low anticancer activity against 5RP7 cell line with an IC_{50} value of 68.33 ± 22.55 μg/mL.

The Cell Proliferation ELISA, BrdU (colorimetric) assay was also performed to quantitate cell proliferation based on the measurement of BrdU incorporation during DNA synthesis in replicating (cycling) cells. Interestingly, the compounds tested in this assay caused DNA synthesis inhibitory effects on A549 cell line, whereas the compounds did not exhibit DNA synthesis inhibitory activity against C6 cell line. This outcome demonstrated the selective DNA synthesis inhibitory effects of the compounds on A549 cells. It can be attributed to the structural differences between A549 human lung adenocarcinoma and C6 rat glioma cell lines.

DNA synthesis inhibitory activity of the compounds against A549 cell line is presented in Fig. 1. DNA synthesis inhibitory effects of the compounds on A549 cell lines revealed the following potency order: Compound **3** > Compound **5** > Compound **2** > Compound **4**.

According to MTT assay, compound **5** can be considered as the most promising anticancer agent due to its significant cytotoxic activity against A549 and C6 cell lines and DNA synthesis inhibitory effect on A549 cell line. The compound exhibited significant anticancer activity against C6 cell line with an IC_{50} value of 11.3 ± 1.2 μg/mL, which was



Scheme 1. The synthetic route for the preparation of the bis-thiazole derivatives (1–10). Reagents and conditions: (i) KSCN, conc. HCl, water, reflux, 3 h; (ii) $ArCOCH_2Br$, ethanol, reflux, 3 h.