Bioorganic Chemistry 53 (2014) 1–7

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Design, synthesis and pharmacological evaluation of novel pyrrolizine derivatives as potential anticancer agents

Ahmed M. Gouda [a,b], Ahmed H. Abdelazeem [a,c], El-Shaimaa A. Arafa [d], Khaled R.A. Abdellatif [e,*]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, Umm Al-Qura University, Mekkah 21955, Saudi Arabia
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia
[d] Department of Pharmacology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[e] Department of Pharmaceutical Organic Chemistry, Beni-Suef University, Beni-Suef 62514, Egypt

ARTICLE INFO

Article history:
Received 7 September 2013
Available online 4 January 2014

Keywords:
Pyrrolizine
Apoptosis
Caspase activation
Breast cancer
Prostate cancer

ABSTRACT

A new series of novel pyrrolizine derivatives has been synthesized and biologically evaluated as potential anticancer agents. The starting compounds, 6-amino-7-cyano-N-(3,5-disubstitutedphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamides **11a–b,** were reacted with different acid chlorides, aldehydes and isocyanates to give the target compounds **12–14**. Structural characterizations of the new compounds were performed using spectral and elemental analysis. All compounds were tested for their anticancer activity against human breast cancer and prostate cancer cell lines, MCF-7 and PC-3 respectively. With exception of compounds **11a** and **13a**, results revealed that all the tested compounds showed half maximal inhibitory concentration (IC_{50}) values less than 40 μM. Compound **12b** and the three urea derivatives **14b–d** showed the most potent anticancer activity with IC_{50} values less than 2.73 μM. The anticancer activity of these compounds was mediated, at least in part, via the induction of apoptosis as indicated by its ability to activate caspase-3/7. In light of the high potency of our novel compounds in targeting both breast and prostate cancers, these compounds warrant continued preclinical development as potential anticancer agents.

1. Introduction

Although many anticancer chemotherapeutic agents have been proven to be successful in the clinical trials, the high rate of mortality presents an urgent need for more safe and more efficient agents [1,2]. The clinical use of many chemotherapeutic agents still associated with many severe adverse reactions and rapid development of resistance despite of the great advances in targeted anticancer therapy [3,4]. The design of anticancer agents targeting cancer cell survival pathways improved the clinical promise of these agents as a component of anticancer therapy [5]. The selective induction of apoptosis in cancer cells is one of the main aims of these promising agents [6]. The activation of caspase-3, the main executioner in apoptosis, was useful in the discovery of many potential anticancer agents [7,8].

The pyrrolizine scaffold constitutes the basic skeleton in many compounds with diverse pharmacological activities [9–11]. Many pyrrolizine derivatives exhibit antitumor activities due to their ability to cross link DNA [12,13]. Recently, several pyrrolizine derivatives were developed lacking the alkylating functions and displayed potent antitumor activities. One of the diphenyl substituted pyrrolizine derivatives that showed a good activity against breast cancer is the selective COX-2 inhibitor, Licofelone **1** [14–16]. The high selectively for COX-2 could play the major role in its anticancer effects [14–16]. In addition, licofelone was found to enhance apoptosis in prostate and colon cancer cells through the mitochondrial pathway [17]. The replacement of the acetic acid moiety of licofelone by additional phenyl acyl side chain significantly increased the anticancer activity, compound **2** (Fig. 1) [17].

On the other hand, many nitrile and aminonitrile derivatives showed anticancer activity through induction of apoptosis and activation of caspase-3 compounds [18]. EpiCept Corporation USA has developed the Anticancer Screening Apoptosis Program (ASAP) that helped in the discovery of the 1-benzoyl-3-cyanopyrrolo

Abbreviations: ASAP, Anticancer Screening Apoptosis Program; $CDCl_3$, deuterated chloroform; DMEM/F-12, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12; DMSO, dimethyl sulfoxide; eV, electron volt; FBS, fetal bovine serum; COX-2, cyclooxygenase-2; IC_{50}, half maximal inhibitory concentration; IR, Infrared spectroscopy; m.p., melting point; *m/z*, mass-to-charge ratio; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; SAR, structural activity relationship.

* Corresponding author. Address: Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt. Fax: +20 (002) 082 2317958.
E-mail address: khaled.ahmed@pharm.bsu.edu.eg (K.R.A. Abdellatif).

0045-2068/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bioorg.2014.01.001

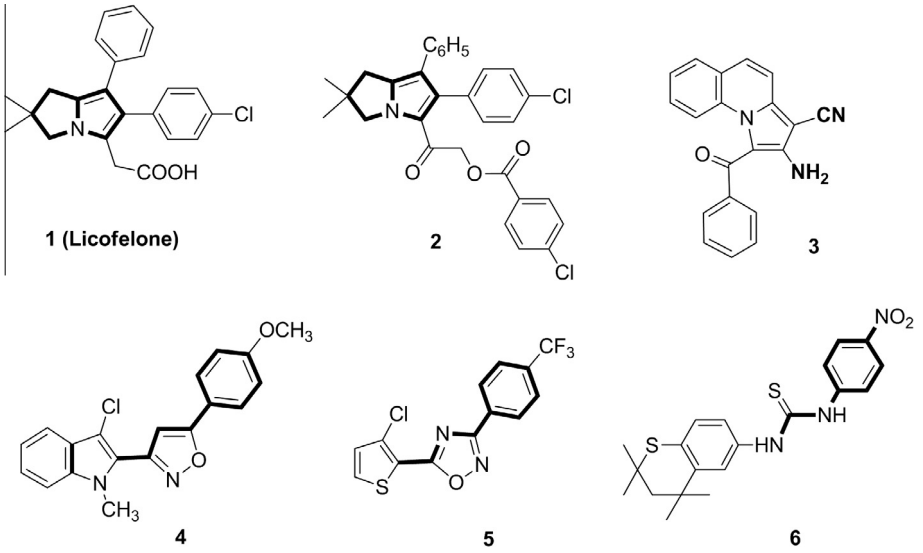

Fig. 1. Structures of some reported apoptosis inducers and caspase activators.

[1,2-a]quinoline **3** as a potent apoptosis inducer [19]. The structural activity relationship (SAR) study of compound **3** revealed that replacing the 3-cyano group by an ester or ketone group led to inactive compounds [18]. Other compounds **4**–**6** sharing certain structural similarity were reported as apoptosis inducers [20–24]. These compounds have a substituted phenyl moiety attached by two or three atoms spacer that contains double bond to another aromatic or heteroaromatic ring. The spacer either consists of sp^2 carbons as in compound **4** or may include one or two nitrogen atoms as in compounds **5** and **6** (Fig. 1). Generally, it was observed that the activities of compounds **1**–**6** vary according to the electronic effect and the position of the monosubstituents where the chloro, methoxy and trifluoromethy substituted derivatives possessed the highest activity [20–24]. In addition, compounds **1**–**2**, **4** and **5** have two phenyl rings attached to the basic nucleus either directly or through three or four atoms spacer.

Promoted by the aforementioned findings and aiming to develop novel and potent anticancer agents, two general scaffolds (**A** and **B,** Fig. 2) incorporating the heterocyclic core, 7-cyano-*N*-(3,5-disubstitutedphenyl)-2,3-dihydro-1H-pyrrolizin-5-carboxamide, were developed. The design concept is based mainly on the structural similarity with compounds **1**–**6** with maximizing the electronic effect of the substituents to investigate their influence on the anticancer activity of the newly designed pyrrolizine derivatives. In order to maximize this electronic effect, we used a disubstituted phenyl ring with two electron withdrawing (dichloro) or electron donating (dimethyl) groups. It was expected that their cumulative effects would be stronger and the impact of these effects on activity would be higher than the monosubstituted pyrrolizine derivatives. Concomitantly, the distance between the additional phenyl ring and pyrrolizine nucleus was varied (two or three atoms), and the substitution pattern of this phenyl ring could affect the target cancer cells and the overall activity.

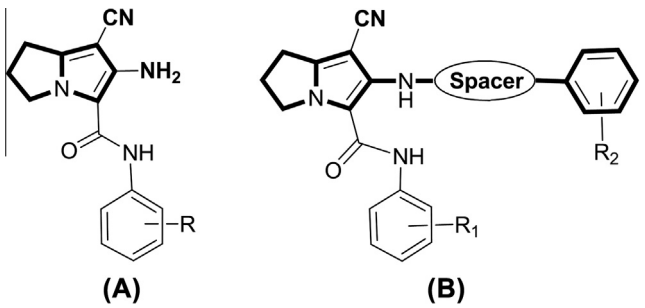

Fig. 2. General scaffolds for the newly designed ligands.

2. Results and discussion

2.1. Chemistry

As shown in **Scheme 1**, preparation of the intermediates **8a**, **8b** and **10** was done according to previously reported procedures [25,26]. Compounds **11a** and **11b** were prepared from the reaction of 2-pyrrolidin-2-ylidine malononitrile **10** with the corresponding α-chloroacetanilide **8a** and **8b** in dry acetone according to previously reported procedures [27]. The reaction proceeded directly via the un-isolated intermediate, *N*-substituted pyrrolidin-2-ylidine malononitrile which cyclized spontaneously by addition of the α-methylene group to one of the two nitrile groups to give compounds **11a**–**b**. Characterization of the newly synthesized compounds was carried out using spectral and elemental analysis. The IR spectrum showed the presence of one sharp absorption band at 2217 and 2220 cm^{-1} assigned for the cyano group in the compounds **11a** and **11b** respectively. On the other hand, the mass spectrum revealed the molecular ions of the compounds **11a** and **11b** at 294 and 335 respectively, and the fragmentation patterns were in concordance with the chemical structure. ^{1}H NMR spectra showed the presence of a singlet proton at 9.09 and 9.29 ppm assigned for the amide protons in the compounds **11a** and **11b** respectively. Further characterization of compound **11a** was done using ^{13}C NMR spectrum as well.

Compounds **11a** and **11b** were used as starting materials for preparing the other target compounds **12**–**14**, as illustrated in Scheme 2. The reaction of compounds **11a** and **11b** with the appropriate acid chloride in dry benzene gave compounds **12a**–**d**. The synthesized compounds were characterized by the spectral and elemental analysis. ^{1}H NMR spectra revealed the presence of two protons at the range of 9.29–10.93 ppm assigned for the amide protons in compounds **12a**–**d**.

Moreover, the condensation of the compounds **11a** and **11b** with the appropriate aldehyde in absolute ethanol in the presence