European Journal of Pharmaceutical Sciences 88 (2016) 166–177

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Chrysin-piperazine conjugates as antioxidant and anticancer agents

Rahul V. Patel [a,f,*], Bhupendra Mistry [b], Riyaz Syed [c], Anuj K. Rathi [d], Yoo-Jung Lee [e], Jung-Suk Sung [e], Han-Seung Shinf [f], Young-Soo Keum [b,*]

[a] Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacky´ University, Šlechtitelu° 27, 783 71 Olomouc, Czech Republic
[b] Organic Research Laboratory, Department of Bioresources and Food Sciences, College of Life and Environmental Sciences, Konkuk University, Seoul, Republic of Korea
[c] Department of Chemistry, J.N.T. University, Kukatpally, Hyderabad 500 085, India
[d] Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University, Šlechtitelù 11, Olomouc, Czech Republic
[e] Department of Life Science, Dongguk University, Goyang, Gyeonggi-do 10326, Republic of Korea
[f] Department of Food Science and Biotechnology, Dongguk University, Biomedical Campus, 32 Dongguk-ro, Ilsandong-gu, Goyang-si, Gyenggi-do, Republic of Korea

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form 30 January 2016
Accepted 20 February 2016
Available online 23 February 2016

Keywords:
Chrysin
Piperazines
Antioxidant
Anticancer
Flavones

ABSTRACT

Synthesis of 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one intermediate treating chrysin with 1,4-dibromobutane facilitated combination of chrysin with a wide range of piperazine moieties which were equipped via reacting the corresponding amines with bis(2-chloroethyl)amine hydrochloride in diethylene glycol monomethyl ether solvent. Free radical scavenging potential of prepared products was analyzed in vitro adopting DPPH and ABTS bioassay in addition to the evaluation of in vitro anticancer efficacies against cervical cancer cell lines (HeLa and CaSki) and an ovarian cancer cell line SK-OV-3 using SRB assay. Bearable toxicity of **7a-w** was examined employing Madin-Darby canine kidney (MDCK) cell line. In addition, cytotoxic nature of the presented compounds was inspected utilizing Human bone marrow derived mesenchymal stem cells (hBM-MSCs). Overall, **7a-w** indicated remarkable antioxidant power in scavenging $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, particularly analogs **7f**, **7j**, **7k**, **7l**, **7n**, **7q**, **7v**, **7w** have shown promising free radical scavenging activity. Analogs **7j** and **7o** are identified to be highly active candidates against HeLa and CaSki cell lines, whereas **7h** and **7l** along with **7j** proved to be very sensitive towards ovarian cancer cell line SKOV-3. None of the newly prepared scaffolds showed cytotoxic nature toward hBM-MSCs cells. From the structure–activity point of view, nature and position of the electron withdrawing and electron donating functional groups on the piperazine core may contribute to the anticipated antioxidant and anticancer action. Different spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analysis (CHN) were utilized to confirm the desired structure of final compounds.

1. Introduction

Reactive oxygen species (ROS) are free radicals carrying unpaired electron in their outer orbit and typically produced during normal aerobic cellular metabolism. ROS play a key role in biological evolution and the origin of life (McCord, 2000), capable of reacting with essential macromolecules thereby introduce damage to vital cell particles, DNA, proteins and lipids and lift the chances of disease processes. There are numbers of ROS, such as superoxide; hydrogen peroxide; a hydroxyl radical; hydroxyl ion; and nitric oxide, which are formed via the addition of electrons implying a sequential reduction of oxygen (Hancock et al., 2001). The imbalance of the equilibrium between oxidant/-antioxidant in support of oxidants is termed as oxidative stress, which plays a role in many pathological conditions, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma (Valko et al., 2007). Natural or synthetic endogenous compounds, responsible for destroying ROS, inhibiting their formation, scavenging their precursors and capable of ROS formation upon binding metal ions are termed as antioxidant (Gilgun-Sherki et al., 2001). Studies support the statement that several antioxidant molecules acquire antitumor, antimicrobial, antiviral and anti-inflammatory effects (Mitscher et al., 1996).

Malignancies figure among the main reasons for deaths and loss of life rate worldwide, with roughly 14 million new cases and 8.2 million cancer-related fatalities in 2012 (IARC, 2014). Over the next two decades, the number of new situations is predicted to rise by about 70% as from 14 million in 2012 to 22 million (WHO, 2015). Among all objectives of cancer research, reactive oxygen species (ROS) play a crucial role in anticancer agent discovery. Since, the creation of extreme ROS outcomes in the launch of cytochrome c from mitochondria (via perturbation of the mitochondrial membrane potential) into the cytosol and

* Corresponding authors.
E-mail addresses: rahul.svnit11@gmail.com (R.V. Patel), rational@konkuk.ac.kr (Y.-S. Keum).

http://dx.doi.org/10.1016/j.ejps.2016.02.011
0928-0987/

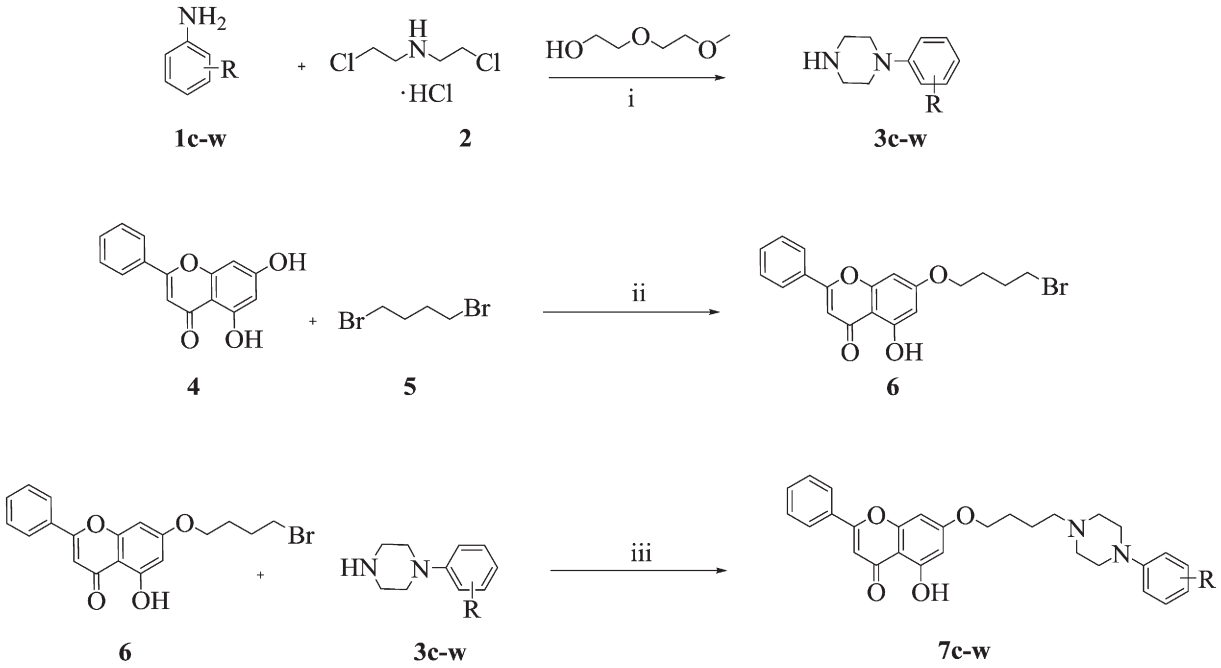
inevitably triggers caspase-9 expression followed by initial of executioner caspases including caspases-3 and -7 which induce execution phase of apoptosis (Simon et al., 2000). Furthermore, initial of caspase-8 is closely engaged in the extrinsic signaling pathway of apoptosis (Jin et al., 2009) which associated with the destruction of NF-κB translocation (Yang et al., 2004). If the action of this aspect is obstructed, tumor cells can go through apoptosis (Dalen and Neuzil, 2003).

Natural products have traditionally been an extremely effective resource for new medicines in all cultures and continue to provide a huge variety of structural layouts for drug discovery and development. Before the post-genomic era and the emergence of high throughput screening, nearly 80% of drug-like molecules rose from natural products or their semisynthetic analogs generated through synthetic modification in the core of natural compounds (Harvey, 2008; Katiyar et al., 2012). Among the extensive variety of natural products, flavonoids are a broad class of polyphenolic secondary metabolites abundant in plants and various common foods. Chrysin, a naturally wide distributed flavonoid, has been revealed to have a plenty of pharmacological actions such as antioxidant (Fonseca et al., 2015; Wang et al., 2014) and anticancer agents (Zhu et al., 2014; Liu et al., 2014). It has a precautionary effect on cancer caused chemically (DMBA-induced hamster buccal pouch carcinomas) as well as on xenograft tumor models by inducing the activity of antioxidant and detoxification enzymes (glutathione peroxidase, glutathione, glutathione reductase, glutathione S-transferase and quinone Reductase), decreasing the actions of cytochrome P450 (CytP450)-dependent monooxygenases, suppressing cellular proliferation, invasion, angiogenesis and inducing apoptosis (Karthikeyan et al., 2013). To be able to provide semi-synthetic derivatives of chrysin, we have chosen piperazine skeletons to link with this flavone moiety because we have successful encounter in previous research with piperazines delivering significant important medicinal results (Patel and Park, 2013).

2. Results and discussion

2.1. Chemistry

The scaffolds rationalized in the present study were prepared to adopt chemical strategies described in Scheme 1. An efficient procedure to prepare *N*-aryl or *N*-heteroaryl piperazines was adopted reacting corresponding anilines (**1a-w**) with bis(2-chloroethyl)amine hydrochloride (**2**) in the presence of diethylene glycol monomethyl ether as described in the literature (Liu and Robichaud, 2005). The analytical data of the synthesized piperazines were of adequate accordance with those reported in the literature (Liu and Robichaud, 2005; Igor et al., 2011). For example, FT-IR data shows aromatic C—H and CC stretching bands at 3077 cm^{-1} and 1584 cm^{-1}, respectively for the compound **3w**. In addition, C—N band corresponding to the piperazine ring appeared at 1305 cm^{-1}. In further analysis, ^1H NMR spectrum of **3w** displayed characteristic signals for the proton atoms of the piperazine ring at 3.52 ppm and 3.27 ppm in the form of multiplets. The types of analysis data were consistent for all other piperazine moieties explored and were further treated in the reaction sequence. In another step of synthetic transformation, intermediate 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**6**) was delivered in 83% of yield reacting readily to available chrysin (**4**) with 1,4-dibromobutane (**5**) under an N_2 atmosphere in the presence of a base (Hu et al., 2011). The OH group observed at 3073 cm^{-1} in the FT-IT spectrum of **6**, whereas carbonyl functionality confirmed upon observing its characteristic band at 1642 cm^{-1}. In the ^1H NMR spectrum of **6**, proton atoms present on the phenyl ring of chrysin core, resonated as multiplet signals in the range 7.88–7.47 ppm, however, the proton of hydroxyl group showed its signal as a singlet at 12.64 ppm. Furthermore, three proton atom signals in the form of the doublet, singlet and doublet at 6.42 ppm, 6.62 ppm and 6.33 ppm attributed to the chromane ring. At last, methylene proton atoms of the aliphatic chain appeared to have their



Reagents & Conditions: **i**. KI (3 mol%), Heat, N_2, 150 °C, 16-48 h; **ii**. K_2CO_3, reflux, 24 h; **iii**. CH_3CN, reflux, 10-38 h.

Scheme 1. Synthesis of piperazine linked chrysin derivatives **7a-w**.