European Journal of Medicinal Chemistry 64 (2013) 529–539

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and biological evaluation of sulforaphane derivatives as potential antitumor agents

Kun Hu, Yan-jie Qi, Juan Zhao, He-fei Jiang, Xin Chen*, Jie Ren*

School of Pharmaceutical Engineering & Life Science, Changzhou University, 1 Gehu Road, Changzhou, Jiangsu 213164, PR China

ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
19 March 2013
Accepted 24 March 2013
Available online 6 April 2013

Keywords:
Sulforaphane
Cytotoxicity
Cell cycle arrest
Apoptosis
Nrf2

ABSTRACT

A series of sulforaphane derivatives were synthesized and evaluated in vitro for their cytotoxicity against five cancer cell lines (HepG2, A549, MCF-7, HCT-116 and SH-SY5Y). The pharmacological results showed that many of the derivatives displayed more potent cytotoxicity than sulforaphane (SFN). Furthermore, SFN and derivative **85** could induce cell cycle arrest at S or G2/M phase and cell apoptosis. SFN and **85** exhibited time- and dose-dependent activation on Nrf2 transcription factor, and **85** acted as a more potent Nrf2 inducer than SFN.

1. Introduction

Isothiocyanates are a class of compounds which are abundant in many cruciferous vegetables, such as broccoli, cauliflower, Brussels sprouts, and cabbage [1,2]. There is increasing epidemiological and clinical evidence that people that intake large amounts of vegetables of the Brassica family, particularly broccoli and other cruciferous vegetables are less likely to develop certain types of cancers [3–5], and thus, many of such analogues containing the isothiocyanates motif have been synthesized for potential medical applications [6]. The isothiocyanates, are hydrolysis products of glucosinolates that are not the putative bioactive compounds in cruciferous vegetables, and the hydrolytic reaction is catalyzed by the endogenous myrosinase which is released by disruption of the plant cell during harvesting, processing, or chewing [7,8].

Sulforaphane (SFN), one of the naturally occurring isothiocyanates, which was first discovered by scientists at Johns Hopkins University in 1992 [9], has attracted considerable research interests due to its important biological and pharmacological properties. SFN is an effective anticancer agent with the ability for both preventing and fighting many types of cancers [10,11]. Early research mainly focused on the “blocking activity” of SFN via Phase 2 enzyme induction, as well as inhibition of enzymes involved in carcinogen activation. In recent years, many studies have showed that SNF could provide protection against tumor development during the post-initiation phase. The mechanisms of SFN suppression effects, including cell cycle arrest and apoptosis, have also been investigated [3,8]. Additionally, pharmacological administration of SFN might be a promising therapeutic approach for cancers treatment. For example, according to a report from the US National Cancer Institute, SFN is considered to be one of the 40 most promising anticancer agents [12–14].

During the past years, a few methods for preparing SFN and its derivatives have been reported in the literature [15–17], and structural modifications have been envisioned to increase their cytotoxicity, especially for their specificity toward tumor cells [18–20]. To the best of our knowledge, there were few reports regarding SFN derivatives that modified the side chain (the methyl group). In our present study, we have designed and synthesized a series of SFN derivatives with different side chains and various lengths of spacers between the isothiocyanato and sulfoxide groups. We evaluated their anticancer activity against several cancer cell lines in vitro, and elucidated the possible mechanism of cell growth inhibition by these SFN derivatives.

2. Chemistry

In order to investigate the structure–activity relationship of the SFN derivatives, we replaced the methyl group in SFN (**77**) with several different alkyl and aryl groups. At the same time, we

---

* Corresponding authors. Tel./fax: +86 519 86334598.
E-mail addresses: xinchen@cczu.edu.cn (X. Chen), renjie2006@163.com (J. Ren).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.03.045

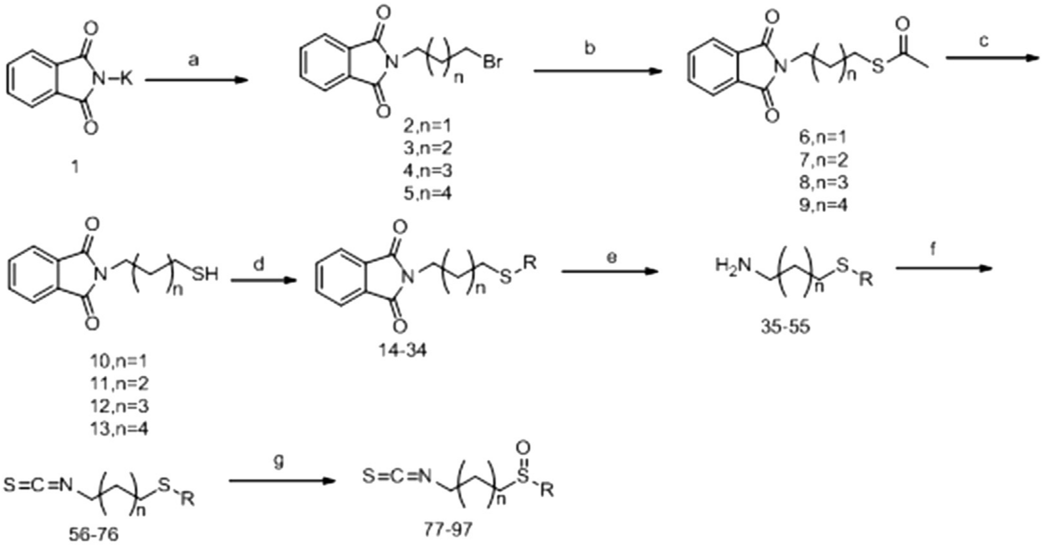
changed the length of spacer between the isothiocyanato and sulfoxide groups. The SFN derivatives (**78**–**97**) were obtained through the synthetic route shown in Scheme 1. Firstly, potassium phthalimide (**1**) was refluxed with different alkyl dibromides in acetone, affording mono-bromides **2**–**5**. Secondly, **2**–**5** were refluxed with potassium thioacetate (KSAc) in THF to give thioacetates **6**–**9** [21,22]. Thiols **10**–**13** were obtained by acidic hydrolysis of **6**–**9** in MeOH. In the following step, **10**–**13** were alkylated with various alkyl bromides in the presence of NaH (method A), affording thio-ethers **14**–**34**. While for phenyl group as the substituent (for synthesizing compound **21**), $Pd_2(dba)_3$ was employed as a catalyst for the alkylation of phenyl bromide (method B) [23]. However, when the same protocol was used for introducing other cyclic and heterocyclic groups, no desired products were obtained. After **14**–**34** were refluxed with 80% hydrazine hydrate in MeOH for 4 h, amines **35**–**55** were produced. By following the known procedure (treated with carbon disulfide, triethylamine and MsCl) [4], isothiocyanates **56**–**76** were obtained in satisfactory yields. Finally, the target compounds **77**–**97** were obtained by oxidizing **56**–**76** with 1 M equiv of MCPBA. When **64** was oxidized by 4 M equiv of MCPBA, sulfone **98** was obtained in 52% yield. The structures of all the final products **77**–**98** were confirmed by their MS, ^{1}H NMR and ^{13}C NMR spectral properties.

3. Results and discussion

3.1. In vitro cytotoxicity

The synthesized SFN derivatives **64**, **78**–**98** were screened for in vitro cytotoxicity against five cancer cell lines, including HepG2 (Liver hepatocellular carcinoma), A549 (human lung adenocarcinoma), MCF-7 (human breast adenocarcinoma), HCT-116 (human colon cancer cell line) and SH-SY5Y (human neuroblastoma cell line), by the standard MTT assay, and using 5-Fu as a positive control. Antitumor potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound, and the results were summarized in Table 1.

As shown in Table 1, all the derivatives exhibited more potent inhibitory activity against HepG2, A549, MCF-7, HCT-116 and SH-SY5Y than SFN, but for A549, HCT-116 and SH-SY5Y cell lines, most of the compounds showed weaker inhibitory activity than 5-Fu. Among the derivatives, **81** and **82** possess significant cytotoxicity against HepG2 with the IC_{50} values at 2.05 μM and 2.16 μM; **82** and **85** had stronger inhibitory activity against MCF-7 with the IC_{50} values at 3.66 μM and 3.30 μM respectively; derivative **91** with the IC_{50} value at 5.64 μM against A549, and derivative **87** with the IC_{50}



14,n=2,R=methyl;
15,n=2,R=ethyl;
16,n=2,R=propyl;
17,n=2,R=n-butyl;
18,n=2,R=iso-propyl;
19,n=2,R=iso-butyl;
20,n=2,R=cyclopentyl;
21,n=2,R=phenyl;
22,n=2,R=benzyl;
23,n=2,R=p-nitrobenzyl;
24,n=2,R=p-methoxybenzyl;
25,n=2,R=phenethyl;
26,n=2,R=2-pyridinylmethyl;
27,n=2,R=2-thienylmethyl;
28,n=2,R=2-furanylmethyl;
29,n=1,R=benzyl;
30,n=1,R=iso-propyl;
31,n=3,R=benzyl;
32,n=3,R=iso-propyl;
33,n=4,R=benzyl;
34,n=4,R=iso-propyl;

35,n=2,R=methyl;
36,n=2,R=ethyl;
37,n=2,R=propyl;
38,n=2,R=n-butyl;
39,n=2,R=iso-propyl;
40,n=2,R=iso-butyl;
41,n=2,R=cyclopentyl;
42,n=2,R=phenyl;
43,n=2,R=benzyl;
44,n=2,R=p-nitrobenzyl;
45,n=2,R=p-methoxybenzyl;
46,n=2,R=phenethyl;
47,n=2,R=2-pyridinylmethyl;
48,n=2,R=2-thienylmethyl;
49,n=2,R=2-furanylmethyl;
50,n=1,R=benzyl;
51,n=1,R=iso-propyl;
52,n=3,R=benzyl;
53,n=3,R=iso-propyl;
54,n=4,R=benzyl;
55,n=4,R=iso-propyl;

56,n=2,R=methyl;
57,n=2,R=ethyl;
58,n=2,R=propyl;
59,n=2,R=n-butyl;
60,n=2,R=iso-propyl;
61,n=2,R=iso-butyl;
62,n=2,R=cyclopentyl;
63,n=2,R=phenyl;
64,n=2,R=benzyl;
65,n=2,R=p-nitrobenzyl;
66,n=2,R=p-methoxybenzyl;
67,n=2,R=phenethyl;
68,n=2,R=2-pyridinylmethyl;
69,n=2,R=2-thienylmethyl;
70,n=2,R=2-furanylmethyl;
71,n=1,R=benzyl;
72,n=1,R=iso-propyl;
73,n=3,R=benzyl;
74,n=3,R=iso-propyl;
75,n=4,R=benzyl;
76,n=4,R=iso-propyl;

77,n=2,R=methyl;
78,n=2,R=ethyl;
79,n=2,R=propyl;
80,n=2,R=n-butyl;
81,n=2,R=iso-propyl;
82,n=2,R=iso-butyl;
83,n=2,R=cyclopentyl;
84,n=2,R=phenyl;
85,n=2,R=benzyl;
86,n=2,R=p-nitrobenzyl;
87,n=2,R=p-methoxybenzyl;
88,n=2,R=phenethyl;
89,n=2,R=2-pyridinylmethyl;
90,n=2,R=2-thienylmethyl;
91,n=2,R=2-furanylmethyl;
92,n=1,R=benzyl;
93,n=1,R=iso-propyl;
94,n=3,R=benzyl;
95,n=3,R=iso-propyl;
96,n=4,R=benzyl;
97,n=4,R=iso-propyl;

Scheme 1. Synthesis of SFN derivatives (**77**–**97**). Reagents and conditions: (a) alkyl dibromide, acetone, reflux, 10 h; (b) KSAc, THF, reflux, 3–5 h; (c) conc. HCl, MeOH, reflux, 3–4 h; (d) method A: NaH, R–Br, THF, rt, 1–2 h; method B: $Pd_2(dba)_3$, DPPF, toluene, bromobenzene, DIPEA, reflux, 2 h; (e) 80% hydrazine hydrate, MeOH, reflux, 3–4 h; (f) (1) Et_3N, CS_2, THF, 0 °C to rt, 90 min; (2) MsCl, 0 °C to rt, 30 min; (g) 85% MCPBA, DCM, 0 °C, 1 h.