Med Chem Res
DOI 10.1007/s00044-014-0929-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antioxidant and antibacterial evaluation of synthetic furomollugin and its diverse analogs

Likai Xia · Akber Idhayadhulla · Yong Rok Lee · Sung Hong Kim · Young-Jung Wee

Received: 5 December 2013 / Accepted: 24 January 2014

Abstract Diverse furomollugin (**3**) and its analogs (**11**–**22**) were synthesized in high yields via ceric ammonium nitrate-catalyzed formal [3 + 2] cycloaddition as a key step. The in vitro antioxidant activities of synthesized compounds were determined by analyzing radical scavenging activities for 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide, and hydrogen peroxide assays. The results showed that the synthesized furomollugin analogs had effective antioxidant power. Dihydronaphthofurans with 2-alkyoxy or 2-aryl group were the most potent radical scavengers in DPPH assay. Moreover, the antibacterial activities of those compounds were also evaluated and the highly active compounds were selected for further determination of minimal inhibitory concentrations (MICs). Compound **19** (MIC = 2 μg/mL) was found to be highly active against the gram-negative bacteria *Escherichia coli* (KCTC-1924) than the Ampicillin standard (MIC = 4 μg/mL). Compound **22** (MIC = 0.5 μg/mL) inhibited gram-positive bacteria *Staphylococcus aureus* (KCTC-1916) growth as effectively as ampicillin (MIC = 0.5 μg/mL).

Keywords CAN · Formal [3 + 2] cycloaddition · Antioxidant · Antibacterial · Furomollugin

L. Xia · A. Idhayadhulla · Y. R. Lee (✉)
School of Chemical Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea
e-mail: yrlee@yu.ac.kr

S. H. Kim
Analysis Research Division, Daegu Center, Korea Basic Science Institute, Taegu 702-701, Republic of Korea

Y.-J. Wee
Department of Food Science and Technology, Yeungnam University, Gyeongsan 712-749, Republic of Korea

Introduction

Dihydronaphthofuran and naphthofuran derivatives have been shown to possess a range of important biological and pharmacological properties (Ho *et al.*, 1996; Srivastava *et al.*, 2006; Le *et al.*, 2009; Matthews *et al.*, 2007 Lumb *et al.*, 2008; Sastry *et al.*, 2010; Chandrashekhar *et al.*, 2011; Shashikala *et al.*, 2011; Kwiecien *et al.*, 2012). Among these, compounds **1**–**3** were isolated from natural sources (Schildknecht and Straub 1976; Inoue *et al.*, 1984; Wu *et al.*, 1991; Otani *et al.*, 2000; Jang *et al.*, 2012). (−)-Nocardione A (**1**) was isolated from the fermentation broth of *Nocardia* sp. TP-A0248 (Otani *et al.*, 2000), which inhibited the activity of Cdc25B, PTP1B, and FAP-1 protein tyrosine phosphatases at a concentration of 10 μM (Fig. 1). It also showed potent antifungal and cytotoxic activities (Otani *et al.*, 2000). Tanshinone I (**2**) was isolated from the roots of *S. miltiorrhiza* and tested for its in vitro fatty acid synthase inhibitory activity. It inhibited the enzyme activity with an IC_{50} value of 12.0 μM (Jang *et al.*, 2012). Mollugin and furomollugin derivatives are biologically interesting compounds isolated from *Galium* species (Inoue *et al.*, 1984). Both mollugin and furomollugin (**3**) were also isolated from the *Rubiaceae* family and have a broad diverse bioactivity (Claessens *et al.*, 2006; Habonimana *et al.*, 2006; Sastry *et al.*, 2010). Especially, furomollugin (**3**) showed cytotoxic activity in human colon carcinoma cells (HT-29) and strongly suppressed HBsAg secretion by human hepatoma Hep3B cells (Schildknecht and Straub 1976; Inoue *et al.*, 1984; Velmurugan *et al.*, 2012). In addition, it has been reported to possess inhibitory activity against DNA topoisomerases I and II (Son *et al.*, 2008). Several synthetic compounds containing a naphthofuran scaffold have been reported to have various biological activities, such as antifungal (Qian *et al.*, 1994),

Published online: 12 February 2014

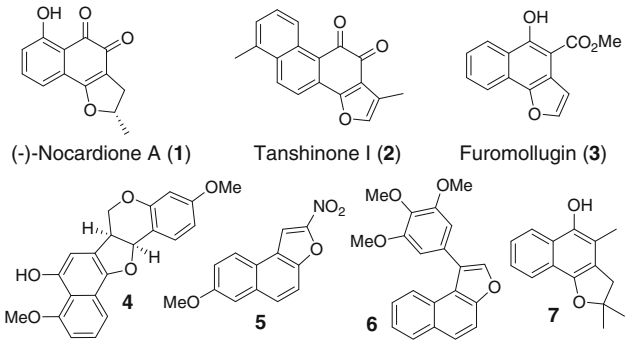


Fig. 1 Naturally occurring and synthetic dihydronaphthofurans and naphthofurans

antibacterial (Einhorn *et al.*, 1984), antiviral (Giovanninetti *et al.*, 1974), *β*-adrenolytic (Gaggi *et al.*, 1982), antitumor (Hranjec *et al.*, 2003; Mahadevan *et al.*, 2001; Mahadevan and Vaidya, 2003), and anthelmintic effects (Rontó *et al.*, 1992; Debnath *et al.*, 1993). Synthetic pterocarpan analog (**4**) showed significant growth inhibitory properties on cells free of HIV-infection ($IC_{50} > 15$ μM) (Engler *et al.*, 1996). 7-Methoxy-2-nitronaphtho[2,1-*b*]furan (**5**) was reported to be one of the strongest mutagens of mammalian cells (Nair *et al.*, 2002). Another synthetic compound **6** showed significant anticancer activity against the human cancer cell lines, COLO 320DM (Colon, human), Caco-2 (Colon, human), and WRL68 (Liver, human) at concentrations of 0.7, 0.65, and 0.50 μg/mL, respectively (Srivastava *et al.*, 2006). Moreover, the α-naphthol derivative, 2,2,4-trimethyl-2,3-dihydronaphtho[1,2-*b*]furan-5-ol (**7**) was known as one of the most active phenolic antioxidants (Barclay *et al.*, 1993, 1995, 1997). Therefore, in the present study, furomollugin (**3**) was chosen as an active structural basis for molecular modification. We have been interested in ceric ammonium nitrate (CAN)-mediated or -catalyzed reactions between 1,3-dicarbonyls or naphthalene-1,4-dione, and olefins, for the preparation of heterocycles (Lee *et al.*, 1998, 2000, 2002, 2003; Lee and Kim, 2001; Xia and Lee, 2013). Using CAN-catalyzed formal [3 + 2] cycloaddition as a key step, we synthesized diverse furomollugin derivatives and evaluated them for antioxidant and antibacterial activities.

Results and discussion

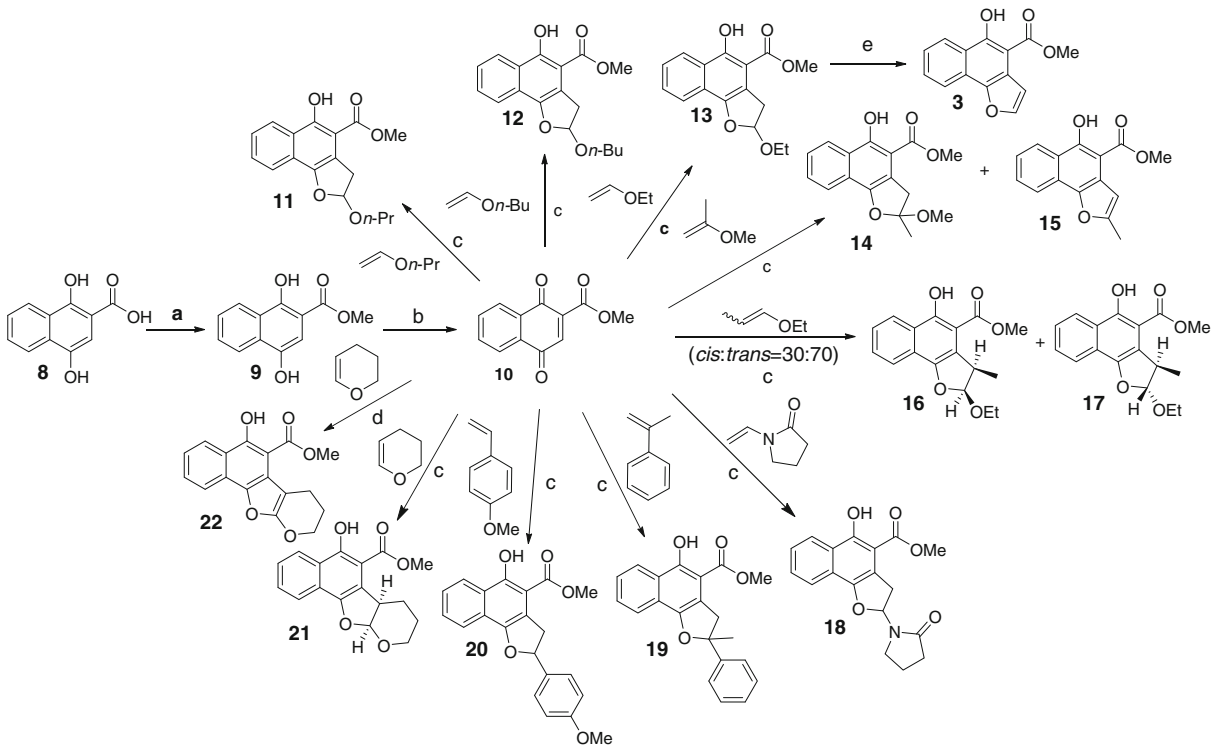
Chemistry

Furomollugin (**3**) and its analogs (**11**–**22**) were synthesized as shown in Scheme 1 (Xia and Lee 2013). The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Our synthesis commences from the commercially available 1,4-dihydroxy-2-naphthoic acid (Sigma-Aldrich, 97 %, **8**). The selective methylation of **8** with MeI in the presence of 2.0 equivalents $NaHCO_3$ in *N*,*N*-dimethylformamide (DMF) at room temperature for 3 h gave methyl 1,4-dihydroxynaphthalene-2-carboxylate (**9**) in 95 % yield (Jacobs *et al.*, 2009). Silver(I) oxide-mediated reaction of **9** afforded methyl 1,4-dioxo-1,4-dihydronaphthalene-2-carboxylate (**10**) in 98 % yield (Brimble *et al.*, 2004). Reactions of **10** with vinyl ethers, such as *n*-propyl vinyl ether, *n*-butyl vinyl ether, and ethyl vinyl ether in the presence of 5 mol% of CAN in acetonitrile at room temperature for 20 min provided the products **11**–**13** in 96, 93, and 95 % yields. However, treatment of **10** with 2-methoxypropene gave compounds **14** (30 %) and **15** (68 %), whereas treatment with ethyl 1-propenyl ether, as a 30:70 mixture of *cis* and *trans*-isomers, provided **16** and **17** in 42 and 44 % yields, respectively. The use of 1-vinyl-2-pyrrolidinone resulted in product **18** in 85 % yield, and α-methylstyrene or 4-methoxystyrene produced **19** and **20** in 94 and 84 % yield, respectively. These compounds were easily separated by column chromatography and identified by spectroscopic analyses. Compound **19** showed a strong IR absorption peak at 1,665 cm^{-1} due to an ester group on the aromatic ring. In ^{1}H-NMR spectrum of **19**, a characteristic methylene peak on the dihydrofuran ring exhibited at δ 3.64 ppm and two singlet peaks showed at δ 3.82 and 1.76 ppm due to a methoxy and a methyl group, respectively. Reaction of **10** with 3,4-dihydro-2*H*-pyran in the presence of 5 mol% of CAN for 0.5 h gave **21** in 95 % yield, but reaction in the presence of two equivalents of CAN provided unexpectedly compound **22** in 85 % yield. The formation of naphthofuran **22** was confirmed by its ^{1}H-NMR spectrum due to the presence of three methylene peaks on the pyranyl ring at δ 4.40 (dd, $J = 5.4$, 5.1 Hz, 2H), 2.81 (dd, $J = 5.4$, 5.1 Hz, 2H), and 2.08–2.01 (m, 2H) ppm. Finally, the elimination of OEt from **13** in the presence of 10 mol% *p*-TsOH in toluene under reflux for 3 h afforded furomollugin (**3**) in 92 % yield.

Biological activity

Antioxidant activity

The synthesized furomollugin (**3**) and its analogs (**11**–**22**) were evaluated for direct scavenging activity against a variety of reactive oxygen and nitrogen species, such as 1,1-diphenyl-2-picrylhyrazyl (DPPH) (Cefarelli *et al.*, 2006; Burits and Bucar, 2000), nitric oxide (NO) (Marcocci *et al.*, 1994), and hydrogen peroxide (H_2O_2) (Ruch *et al.*, 1989). Free radical scavenging activities are expressed as percent inhibitions, and the results are presented in Tables 1, 2 and 3. All synthesized compounds showed moderate to good



Scheme 1 Synthesis of furomollugin (**3**) and its analogs (**11**–**22**). Reagents and conditions: *a* Mel, (1.0 equiv), $NaHCO_3$, (2.0 equiv), DMF, RT, 3 h, 95 %. *b* Ag_2O, (3.3 equiv), $MgSO_4$, (2.0 equiv), Et_2O, RT, 16 h. *c* Olefin (2.0 equiv), catalyst CAN (0.5 equiv), acetonitrile, RT, 20 min, (**11**–**21**, 30–96 %). *d* 3,4-Dihydro-2H-pyran (2.0 equiv), CAN (2.0 equiv), acetonitrile, RT, 30 min, (**22**, 85 %). *e* *p*-Toluenesulfonic acid (PTSA, 0.1 equiv), toluene, reflux, 7 h, (**3**, 92 %)

scavenging activities in DPPH, NO, and H_2O_2 assays. Compounds **11**–**14**, **16**, **18**, and **20**–**21**, showed high DPPH free radical scavenging activities (67–85 %, at 100 μg/mL), whereas compounds **15**, **17**, **19**, and **22** showed low DPPH reducing activities (37–56 %, at 100 μg/mL). Compounds **16** and **18** have potent NO scavenging activities (56 and 66 % at 100 μg/mL, respectively), but these were lower than that of Trolox (88 % at 100 μg/mL). All other compounds

Table 1 The in vitro DPPH radical scavenging activity of furomollugin (**3**) and its analogs (**11**–**22**)

Compounds	Concentration (μg/mL)[a]				IC_{50} (μg/mL)
	25	50	75	100	
3	24.2 ± 0.36	30.3 ± 1.89	35.2 ± 0.22	41.1 ± 1.54	>100
11	59.2 ± 0.56	61.0 ± 1.46	66.2 ± 1.77	68.1 ± 1.01	19.2 ± 0.16
12	67.2 ± 1.36	69.2 ± 1.98	71.2 ± 1.47	73.0 ± 1.16	18.2 ± 0.06
13	61.1 ± 0.15	64.2 ± 1.36	66.2 ± 0.26	71.9 ± 1.12	20.8 ± 0.23
14	78.8 ± 1.08	80.6 ± 0.16	82.8 ± 2.16	84.9 ± 1.65	13.4 ± 0.33
15	31.2 ± 1.41	34.1 ± 0.19	36.0 ± 1.59	37.0 ± 1.65	>100
16	61.1 ± 0.15	66.1 ± 0.19	72.4 ± 0.66	80.8 ± 1.36	17.2 ± 0.15
17	39.5 ± 0.96	42.1 ± 1.62	45.1 ± 0.15	55.8 ± 0.97	84.7 ± 0.22
18	51.1 ± 1.69	56.3 ± 0.26	62.3 ± 1.09	69.7 ± 1.20	21.2 ± 0.26
19	35.3 ± 0.56	39.8 ± 1.16	49.2 ± 1.41	53.7 ± 1.47	87.5 ± 0.29
20	61.2 ± 0.98	65.2 ± 1.77	70.2 ± 1.49	76.9 ± 1.06	16.2 ± 0.31
21	51.1 ± 1.39	57.4 ± 1.95	61.2 ± 1.16	67.5 ± 0.65	22.7 ± 0.65
22	24.1 ± 1.69	31.3 ± 1.06	36.2 ± 1.05	39.9 ± 0.45	>100
Trolox	72.9 ± 1.27	80.1 ± 1.41	82.8 ± 0.36	84.1 ± 0.55	15.0 ± 0.23

[a] Value were the means of three replicates ± SD

Table 2 The in vitro nitric oxide (NO) scavenging activity of furomollugin (**3**) and its analogs (**11–22**)

Compounds	Concentration (μg/mL)[a]				IC_{50} (μg/mL)
	25	50	75	100	
3	21.2 ± 1.64	27.0 ± 1.00	30.2 ± 0.74	32.7 ± 0.95	>100
11	26.8 ± 1.10	30.3 ± 0.18	36.2 ± 1.15	41.2 ± 0.21	>100
12	10.3 ± 1.49	14.6 ± 0.11	20.3 ± 0.18	26.6 ± 0.65	>100
13	34.2 ± 0.98	39.6 ± 0.44	42.7 ± 0.24	48.9 ± 1.30	>100
14	NA	06.0 ± 1.19	10.3 ± 0.18	15.2 ± 1.56	>100
15	NA	NA	5.0 ± 0.15	7.5 ± 1.45	>100
16	44.9 ± 1.98	47.9 ± 1.60	50.6 ± 1.11	55.8 ± 1.20	50.7 ± 0.19
17	NA	NA	1.1 ± 1.19	5.6 ± 0.12	>100
18	42.4 ± 0.68	55.4 ± 0.70	57.6 ± 0.79	66.1 ± 1.56	39.4 ± 0.11
19	NA	NA	NA	5.3 ± 1.14	>100
20	15.3 ± 0.36	20.1 ± 1.06	24.5 ± 0.19	28.6 ± 0.65	>100
21	11.3 ± 0.89	15.7 ± 1.26	21.8 ± 1.44	26.6 ± 0.85	>100
22	NA	5.4 ± 1.29	10.7 ± 0.55	14.1 ± 1.74	>100
Trolox	80.1 ± 0.61	82.2 ± 0.27	84.7 ± 0.57	87.5 ± 1.47	12.0 ± 1.32

[a] Value were the means of three replicates ± SD, NA (Not active)

Table 3 The in vitro hydrogen peroxide (H_2O_2) scavenging activity of furomollugin (**3**) and its analogs (**11–22**)

Compounds	Concentration (μg/mL)[a]				IC_{50} (μg/mL)
	25	50	75	100	
3	30.3 ± 1.12	36.2 ± 0.49	40.7 ± 0.29	45.3 ± 0.12	>100
11	12.7 ± 0.25	18.6 ± 1.48	21.7 ± 1.55	27.4 ± 0.25	>100
12	NA	NA	1.3 ± 0.21	4.1 ± 1.26	>100
13	NA	NA	1.59	3.5 ± 0.24	>100
14	33.7 ± 1.69	38.54 ± 0.69	41.3 ± 1.29	46.6 ± 0.65	>100
15	NA	NA	NA	2.4 ± 0.48	>100
16	NA	NA	NA	0.1 ± 0.65	>100
17	NA	NA	NA	1.1 ± 0.03	>100
18	NA	NA	NA	3.5 ± 0.65	>100
19	58.3 ± 1.09	61.2 ± 1.54	68.3 ± 0.12	70.3 ± 1.56	18.2 ± 0.26
20	0.3 ± 0.29	8.9 ± 1.47	10.6 ± 0.94	13.3 ± 0.26	>100
21	NA	NA	1.6 ± 0.24	3.5 ± 0.65	>100
22	26.2 ± 0.44	36.8 ± 0.36	42.7 ± 1.69	54.8 ± 0.36	91.2 ± 1.84
Trolox	65.2 ± 1.27	68.1 ± 1.41	70.3 ± 0.36	74.2 ± 0.55	16.0 ± 1.23

NA not active

[a] Value were the means of three replicates ± SD

were weak NO scavengers (5–49 %, at 100 μg/mL). In the H_2O_2 assay, compound **19** was found to possess significant free radical scavenging activity (70 % at 100 μg/mL) as compared with Trolox (74 %, at 100 μg/mL), whereas the other compounds **3**, **11**, **14**, and **22** showed moderate inhibitory activity (27–55 %, at 100 μg/mL). The remaining compounds were very weak H_2O_2 scavengers. It is worth noting that comparable free radical scavenging activities were observed for all compounds tested at a concentration of 25 μg/mL. Importantly, compound **14** showed excellent antioxidant activity with an IC_{50} value of 13.4 μg/mL in DPPH assay, whereas the IC_{50} value of the Trolox standard was 15.0 μg/mL. Compound **18** showed high nitric oxide (NO) scavenging activity with an IC_{50} value of 39.4 μg/mL, whereas the IC_{50} value of the Trolox standard was 12.0 μg/mL. In hydrogen peroxide (H_2O_2) assay, Compound **19** exhibited

potent scavenging activity with an IC_{50} value of 18.2 μg/mL, whereas the IC_{50} value of the Trolox standard was 16.0 μg/mL.

The present study also revealed that the synthesized furomollugin (**3**) and its analogs (**11**–**22**) were more effective in the DPPH assay than NO and H_2O_2 assays. In DPPH assay, the 5-hydroxy and naphthofuran moiety on the furomollugin analogs have significant influence on their radical scavenging activity. A 5-hydroxy group on a furomollugin skeleton is essential to exhibit the antioxidant activity. The synthesized furomollugin analogs bearing 2-alkyoxy group or 2-aryl, such as compounds **14** and **20**, have more effective antioxidant powers which are similar to the well-known phenolic antioxidant **7**. The results could be explained by the strong electron-donating inductive effect on the 2,3-dihydronaphtho[1,2-*b*]furans which will stabilize the phenolic radicals (Barclay *et al.*, 1995). On the other hand, the order of inhibitory activity of **14** > **13** > **21** > **19**, based on their IC_{50} values, is also largely explainable in terms of stereoelectronic constraints, and thus reflects their intrinsic antioxidant properties (Burton *et al.*, 1985). Interestingly, *cis*-**16** exhibited much higher antioxidant activity than *trans*-**17** isomer for steric reasons (Burton *et al.*, 1985). Finally, naphtho[1,2-*b*]furans **3**, **15**, and **22** showed very poor radical scavenging activities because of the combination of electron inductive effects and stereoelectronic constraints as mentioned above.

Antibacterial evaluation

Antibacterial activities of furomollugin (**3**) and its analogs **11**–**22** were screened using the diffusion disk method. The experiments were performed using test bacterial organisms belonging to the gram-negative and gram-positive groups, namely, *Escherichia coli* (*E. coli*, KCTC-1924) and *Staphylococcus aureus* (*S. aureus*, KCTC-1916), respectively.

Escherichia coli, a typical gram-negative bacterium, can be grown easily in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* is high sensitivity for antibiotics. Thus, it is most widely studied prokaryotic model organism, and it is an important species in the fields of biotechnology and microbiology. On the other hand, *S. aureus*, a common gram-positive bacterium, which is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Moreover, both *E. coli* and *S. aureus* are commercially available from the Korean Collection for Type Cultures (KCTC) in the Korea Research Institute of Bioscience and Biotechnology (KRIBB). For all the reasons mentioned above, we focused on *E. coli* (KCTC-1924) and *S. aureus* (KCTC-1916) in our studies.

Zone of inhibition was determined for **3** and **11**–**22**, and the results are summarized in Table 4 (Bauer *et al.*, 1966). Against *E. coli* at a concentration of 100 μg/mL, compound **19** was found to be highly active (18 mm zone of inhibition) using Ampicillin as a positive control (17 mm zone of inhibition), whereas **3**, **14**–**18**, and **20**–**22** exhibited moderate activity (11–15 mm zone of inhibition). Compounds **11** and **13** showed weak inhibitory activities, and compound **12** exhibited no activity. Against *S. aureus* at a concentration of 100 μg/mL, compounds **21** (20 mm zone of inhibition) and **22** (23 mm zone of inhibition) were equally active as Ampicillin (23 mm zone of inhibition), whereas compounds **3**, **11**–**16**, **19**, and **20** displayed moderate activity (10–15 mm zone of inhibition), and compounds **17** and **18** exhibited weak or no activity, respectively, against *S. aureus.*

Table 4 Antibacterial activities of furomollugin (**3**) and its analogs (**11**–**22**)

Compounds	Diameter of growth inhibition zone (mm)	
	Escherichia coli (Gram-negative)	*Staphylococcus aureus* (Gram-positive)
Control: DMSO	0.0	0.0
St: Ampicillin	17	23
3	13	11
11	8	10
12	NA	13
13	10	12
14	11	15
15	11	15
16	13	11
17	13	8
18	14	NA
19	18	12
20	14	10
21	15	20
22	13	23

NA not active

The highly active compounds (**14**, **15**, **19**, **21**, and **22**) were selected for further determination of minimal inhibitory concentrations (MICs), as shown in Table 5. The results were in accordance with the results obtained in the primary screening. It would be concluded that compound **19** (MIC = 2 μg/mL) was more active against *E. coli* than Ampicillin standard (MIC = 4 μg/mL). In addition, compound **22** (MIC = 0.5 μg/mL) showed equipotential activity against *S. aureus* with respect to the standard drug Ampicillin (MIC = 0.5 μg/mL).

Conclusions

In summary, we described the synthesis of diverse furomollugin (**3**) and its analogs **11**–**22**, and screened for their antioxidant and antibacterial activities. Those compounds

Table 5 The minimal inhibitory concentrations (MIC, μg/mL) of selected compounds

Compound no.	Minimal inhibitory concentration (MIC, μg/mL)	
	E. coli	*S. aureus*
14	ND	64
15	ND	64
19	2	ND
21	64	2
22	ND	0.5
Ampicillin	4	0.5

ND not determined

were more effective in the DPPH assay than NO and H_2O_2 assays. Importantly, compounds **14** and **20** showed excellent DPPH scavenging activities, which were almost as efficient as the Trolox standard. Comparing with the Trolox standard, compounds **16** and **18** showed decent radical scavenging activities in NO assay, and compound **19** showed potent radical scavenging activity in H_2O_2 assay. Most of compounds were active in vitro evaluation of their antibacterial activities. Importantly, compound **19** was more active against *E. coli* bacterial strain than the standard drug Ampicillin. Compound **22** was highly active against the *S. aureus* bacterial strain at a level of Ampicillin. Our results suggest that these substances represent interesting lead compounds for the development of novel antioxidant and antibacterial agents.

Experimental

All chemicals were purchased from the Sigma-Aldrich, Fluka, or Tokyo Chemical Industry, and used without further purification. Solvents were dried and distilled prior to use. All synthetic experiments were carried out in a N_2 atmosphere. *Merck* precoated silica gel plates (Art. *5554*) with a fluorescent indicator were used for analytical TLC. Flash column chromatography was performed using silica gel 7734 (Merck). The ^{1}H and ^{13}C NMR spectra were recorded on a *Bruker Model ARX* (300 and 75 MHz, resp.) spectrometer using TMS as internal standard in deuterated chloroform or dimethylsulfoxide. The IR spectra were recorded on a *BioRad FTS 3000* spectrophotometer. The HRMS were carried out at the Korea Basic Science Institute.

Synthesis

*General procedure for the preparation of dihydronaphtho[1,2-b]furan derivatives **11–21***

To a solution of methyl 1,4-dihydroxynaphthalene-2-carboxylate (**9**, 218 mg, 1.0 mmol) and the corresponding olefin (2.0 mmol) in MeCN (5.0 mL) was added cerium (IV) ammonium nitrate (CAN, 5.0 mol%) at room temperature. The reaction was monitored by TLC. After all starting material had been consumed, water (10 mL) was added, and the solution was extracted with ethyl acetate (10 mL × 3). Evaporation of the solvent and purification by silica gel column chromatography using hexane–ethyl acetate (10:1) as eluant gave the desired products.

*Methyl 5-hydroxy-2-propoxy-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**11**)*

Yellow liquid; yield 290 mg, 96 %; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.82 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.4, 7.2 Hz, 1H), 7.49 (dd, J = 8.4, 7.2 Hz, 1H), 5.85–5.82 (m, 1H), 3.94 (s, 3H), 3.64–3.52 (m, 3H), 3.45–3.38 (m, 1H), 1.76–1.55 (m, 2H), 0.94 (dd, J = 7.5, 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.51, 156.18, 145.63, 129.05, 125.38, 124.39, 124.30, 121.06, 115.06, 106.02, 103.03, 100.76, 70.17, 52.03, 40.18, 22.84, 10.66; IR (neat): 3,066, 2,961, 2,877, 1,663, 1,448, 1,351, 1,235, 1,162, 1,104, 1,016, 897, 768 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{17}H_{18}O_5$: 302.1154, Found 302.1151.

*Methyl 2-butoxy-5-hydroxy-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**12**)*

Yellow liquid; yield 294 mg, 93 %; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.78 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.58 (dd, J = 8.1, 7.2 Hz, 1H), 7.46 (dd, J = 8.1, 7.2 Hz, 1H), 5.80 (dd, J = 6.6, 2.4 Hz, 1H), 3.91 (s, 3H), 3.62–3.50 (m, 3H), 3.41–3.34 (m, 1H), 1.61–1.49 (m, 2H), 1.39–1.32 (m, 2H), 0.94–0.87 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.52, 156.19, 145.64, 129.05, 125.38, 124.31, 124.26, 121.06, 115.06, 106.02, 103.04, 100.76, 68.25, 52.03, 40.18, 31.61, 19.38, 13.78; IR (neat): 3,423, 3,065, 2,954, 2,871, 1,663, 1,599, 1,447, 1,350, 1,235, 1,162, 1,091, 1,018, 896, 767, 646 cm^{-1}; HRMS (EI$^+$): *m/z*: calcd for $C_{18}H_{20}O_5$: 316.1311, Found 316.1312

*Methyl 2-ethoxy-5-hydroxy-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**13**)*

Yellow solid; yield 274 mg, 95 %; mp 81–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.75 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.56 (dd, J = 8.4, 8.1 Hz, 1H), 7.45 (dd, J = 8.4, 8.1 Hz, 1H), 5.78 (d, J = 6.6 Hz, 1H), 4.05–3.94 (m, 1H), 3.88 (s, 3H), 3.72–3.62 (m, 1H), 3.48 (dd, J = 17.7, 6.3 Hz, 1H), 3.33 (dd, J = 18.0, 2.4 Hz, 1H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.37, 156.11, 145.55, 128.94, 125.27, 124.37,

124.25, 124.18, 121.00, 115.00, 105.82, 102.95, 63.82, 51.87, 40.13, 15.09; IR (KBr): 3,062, 2,974, 1,661, 1,599, 1,446, 1,349, 1,236, 1,162, 1,100, 1,017, 946, 896, 767, 646 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{16}H_{16}O_5$: 288.0998, Found 288.0998.

*Methyl 5-hydroxy-2-methoxy-2-methyl-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**14**)*

Yellow solid; yield 86 mg, 30 %; mp 160–161 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 11.80 (s, 1H), 8.36 (d, $J = 8.4$ Hz, 1H), 7.92 (d, $J = 8.4$ Hz, 1H), 7.60 (dd, $J = 8.1$, 7.2 Hz, 1H), 7.48 (dd, $J = 8.1$, 7.2 Hz, 1H), 3.96 (s, 3H), 3.61 (d, $J_{AB} = 18.0$ Hz, 1H), 3.44 (d, $J_{AB} = 18.0$ Hz, 1H), 3.32 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.55, 156.17, 145.93, 129.15, 125.52, 124.54, 124.36, 123.94, 121.16, 115.32, 111.39, 102.87, 52.07, 49.66, 43.79, 24.63; IR (KBr): 3,449, 2,946, 1,656, 1,598, 1,445, 1,381, 1,344, 1,281, 1,236, 1,160, 1,019, 860, 777, 731 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{16}H_{16}O_5$: 288.0998, Found 288.0994.

*Methyl 5-hydroxy-2-methylnaphtho[1,2-b]furan-4-carboxylate (**15**)*

White solid; yield 174 mg, 68 %; mp 135–136 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 12.19 (s, 1H), 8.41 (d, $J = 8.1$ Hz, 1H), 8.11 (d, $J = 8.1$ Hz, 1H), 7.65 (dd, $J = 8.1$, 7.2 Hz, 1H), 7.45 (dd, $J = 8.1$, 7.2 Hz, 1H), 6.77 (s, 1H), 4.04 (s, 3H), 2.52 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.02, 158.74, 154.85, 143.38, 129.79, 124.78, 124.51, 124.27, 121.97, 121.19, 119.33, 105.47, 98.99, 52.11, 14.11; IR (KBr): 3,450, 2,953, 2,940, 1,646, 1,578, 1,443, 1,343, 1,241, 1,161, 1,095, 1,044, 987, 776 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{15}H_{12}O_4$: 256.0,736, Found 256.0737.

*cis-Methyl 2-ethoxy-5-hydroxy-3-methyl-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**16**)*

Yellow solid; yield: 127 mg, 42 %; mp 103–104 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 11.89 (s, 1H), 8.35 (d, $J = 8.4$ Hz, 1H), 7.89 (d, $J = 7.8$ Hz, 1H), 7.58 (dd, $J = 8.1$, 7.2 Hz, 1H), 7.46 (dd, $J = 8.1$, 7.2 Hz, 1H), 5.83 (d, $J = 6.6$ Hz, 1H), 4.15–4.07 (m, 1H), 3.97 (s, 3H), 3.84–3.72 (m, 2H), 1.35 (t, $J = 7.2$ Hz, 3H), 1.20 (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.44, 156.37, 144.11, 129.11, 125.65, 124.62, 124.38, 124.28, 121.41, 109.61, 102.62, 66.36, 52.05, 43.01, 42.63, 15.23, 13.60; IR (KBr): 2,974, 1,655, 1,443, 1,349, 1,233, 1,087, 916, 767 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{17}H_{18}O_5$: 302.1,154, Found 302.1156.

*trans-Methyl 2-ethoxy-5-hydroxy-3-methyl-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**17**)*

Yellow solid; yield: 133 mg, 44 %; mp 112–113 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 11.98 (s, 1H), 8.38 (d, $J = 8.4$ Hz, 1H), 7.93 (d, $J = 8.1$ Hz, 1H), 7.60 (dd, $J = 8.4$, 8.1 Hz, 1H), 7.48 (dd, $J = 8.4$, 8.1 Hz, 1H), 5.40 (brs, 1H), 4.03–3.98 (m, 1H), 3.97 (s, 3H), 3.73–3.65 (m, 2H), 1.28 (d, $J = 6.9$ Hz, 3H), 1.22 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.45, 156.90, 145.23, 129.20, 125.57, 124.79, 124.77, 124.48, 121.17, 121.09, 111.95, 102.65, 63.85, 52.01, 46.46, 18.38, 15.18; IR (KBr): 2,970, 1,657, 1,444, 1,355, 1,234, 1,101, 910, 766 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{17}H_{18}O_5$: 302.1154, Found 302.1155.

*Methyl 5-hydroxy-2-(2-oxopyrrolidin-1-yl)-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**18**)*

Yellow solid; yield: 278 mg, 85 %; mp 173–174 °C. IR (KBr): 3,055, 2,984, 1,694, 1,448, 1,235, 1,163, 1,094, 1,016, 890, 773, 652 cm^{-1}. ^1H-NMR (300 MHz, $CDCl_3$) δ 11.81 (s, 1H), 8.36 (d, $J = 8.4$ Hz, 1H), 7.87 (d, $J = 8.1$ Hz, 1H), 7.60 (dd, $J = 8.1, 6.9$ Hz, 1H), 7.49 (dd, $J = 8.4, 6.9$ Hz, 1H), 6.77 (dd, $J = 9.3$, 4.5 Hz, 1H), 3.98 (s, 3H), 3.77 (dd, $J = 18.0$, 9.6 Hz, 1H), 3.38 (dd, $J = 17.7$, 4.2 Hz, 1H), 3.27–3.20 (m, 2H), 2.48–2.42 (m, 2H), 2.04–1.94 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 175.42, 171.32, 156.38, 146.19, 129.38, 125.83, 124.57, 124.31, 123.51, 121.22, 113.86, 102.46, 83.46, 52.24, 41.17, 36.16, 31.17, 17.71. HRMS (EI^+): *m/z*: calcd for $C_{18}H_{17}NO_5$: 327.1107, Found 327.1103.

*Methyl 5-hydroxy-2-methyl-2-phenyl-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**19**)*

Yellow solid; Yield: 314 mg, 94 %; mp 147–148 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 11.72 (s, 1H), 8.28 (d, $J = 8.4$ Hz, 1H), 7.96 (d, $J = 8.4$ Hz, 1H), 7.55–7.50 (m, 1H), 7.45–7.37 (m, 3H), 7.27–7.22 (m, 2H), 7.17–7.12 (m, 1H), 3.82 (s, 3H), 3.64 (s, 2H), 1.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.63, 156.05, 147.26, 146.42, 129.12, 128.33, 126.98, 125.51, 124.55, 124.41, 124.37, 124.23, 121.33, 115.36, 103.25, 88.76, 52.07, 48.53, 29.32; IR (KBr): 3,420, 3,060, 3,026, 2,954, 1,665, 1,646, 1,597, 1,449, 1,379, 1,350, 1,243, 1,161, 1,066, 1,020, 863, 769, 700, 642 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{21}H_{18}O_4$: 334.1205, Found 334.1205.

*Methyl 5-hydroxy-2-(4-methoxyphenyl)-2,3-dihydronaphtho[1,2-b]furan-4-carboxylate (**20**)*

Yellow solid; yield 294 mg, 84 %; mp 126–127 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 11.85 (s, 1H), 8.38 (d,

$J = 8.4$ Hz, 1H), 7.94 (d, $J = 8.1$ Hz, 1H), 7.59 (dd, $J = 8.1$, 6.9 Hz, 1H), 7.49 (dd, $J = 8.4$, 6.9 Hz, 1H), 7.37 (d, $J = 8.7$ Hz, 2H), 6.90 (d, $J = 8.7$ Hz, 2H), 5.79 (dd, $J = 9.3$, 8.7 Hz, 1H), 3.92–3.83 (m, 4H), 3.79 (s, 3H), 3.51–3.43 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.54, 159.30, 155.97, 147.13, 134.42, 129.05, 127.16, 125.46, 124.45, 124.26, 123.92, 121.27, 115.77, 113.89, 103.06, 83.73, 55.20, 52.00, 41.91; IR (KBr): 3,060, 3,005, 2,952, 2,836, 1,660, 1,514, 1,447, 1,348, 1,297, 1,239, 1,169, 1,089, 1,017, 832, 770, 644 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{21}H_{18}O_5$: 350.1154, Found 350.1154

*Methyl 5-hydroxy-7,8,9,10a-tetrahydro-6bH-naphtho[2′,1′:4,5]furo[2,3-b]pyran-6-carboxylate (**21**)*

Yellow solid; yield 285 mg, 95 %; mp 166–167 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.92 (s, 1H), 8.36 (d, $J = 8.4$ Hz, 1H), 7.94 (d, $J = 8.1$ Hz, 1H), 7.60 (dd, $J = 8.4$, 8.1 Hz, 1H), 7.49 (dd, $J = 8.4$, 8.1 Hz, 1H), 5.99 (d, $J = 6.3$ Hz, 1H), 3.99 (s, 3H), 3.95–3.92 (m, 2H), 3.53–3.46 (m, 1H), 2.22–2.15 (m, 1H), 1.78–1.65 (m, 1H), 1.63–1.54 (m, 1H), 1.45–1.32 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.36, 156.46, 144.52, 129.36, 125.83, 124.65, 124.56, 124.34, 121.57, 121.47, 105.29, 102.35, 62.13, 52.20, 40.22, 26.10, 21.55; IR (KBr): 3,451, 2,958, 2,872, 1,652, 1,591, 1,446, 1,388, 1,349, 1,240, 1,156, 1,072, 1,021, 949, 863, 771 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{17}H_{16}O_5$: 300.0998, Found 300.0997.

*Methyl 5-hydroxy-8,9-dihydro-7H-naphtho[2′,1′:4,5]furo[2,3-b]pyran-6-carboxylate (**22**)*

To a solution of methyl 1,4-dioxo-1,4-dihydronaphthalene-2-carboxylate (**6a**, 216 mg, 1.0 mmol) and 3,4-dihydro-2*H*-pyran (168 mg, 2.0 mmol) in MeCN (10.0 mL) was added CAN (1.10 g, 200 mol%) at room temperature. The progress of the reaction was monitored by TLC. After all the starting material was consumed, water (10 mL) was added and the solution was extracted with ethyl acetate (10 mL × 3). Evaporation of the solvent and purification by column chromatography on silica gel using hexane–ethyl acetate (10:1) gave **22** (253 mg, 85 %) as a yellow solid. MP: 193–194 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.37 (s, 1H), 8.35 (d, $J = 8.4$ Hz, 1H), 7.99 (d, $J = 8.4$ Hz, 1H), 7.59 (dd, $J = 7.8$, 7.5 Hz, 1H), 7.35 (dd, $J = 7.8$, 7.5 Hz, 1H), 4.40 (dd, $J = 5.4$, 5.1 Hz, 2H), 3.97 (s, 3H), 2.81 (t, $J = 6.3$ Hz, 2H), 2.08–2.01 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.84, 159.65, 158.96, 129.91, 124.61, 124.34, 123.45, 122.15, 121.38, 120.54, 118.86, 99.43, 88.71, 69.37, 51.63, 23.23, 21.68; IR (KBr): 3,444, 3,069, 2,951, 2,863, 1,650, 1,619, 1,579, 1,442, 1,365, 1,336, 1,249, 971, 809, 774 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{17}H_{14}O_5$: 298.0841, Found 298.0843.

*Furomollugin (**3**)*

To a solution of dihydronaphtho[1,2-*b*]furan **13** (144 mg, 0.50 mmol) in an anhydrous toluene (10.0 mL) was added *p*-toluenesulfonic acid (PTSA, 18 mg, 0.10 mmol) at room temperature. The reaction mixture was refluxed for 7 h. After cooling to room temperature, the solution was treated with saturated $NaHCO_3$ solution and extracted with EtOAc (3 × 10.0 mL). The organic layers were combined and dried over an anhydrous $MgSO_4$. After removal of the drying agent and the solvent, the residue was purified by column chromatography on silica gel using hexane–ethyl acetate (10:1) to yield **3** (111 mg, 92 %) as a yellow solid. MP: 114–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.18 (s, 1H), 8.37 (d, $J = 8.7$ Hz, 1H), 8.08 (d, $J = 8.4$ Hz, 1H), 7.63 (dd, $J = 8.1$, 7.2 Hz, 1H), 7.61–7.59 (m, 1H), 7.46 (dd, $J = 8.1$, 7.2 Hz, 1H), 7.00 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.73, 158.99, 144.05, 143.98, 129.78, 124.81, 124.69, 124.58, 122.64, 119.55, 119.43, 109.04, 98.92, 52.03; IR (KBr): 3,121, 3,092, 2,947, 1,671, 1,638, 1,575, 1,439, 1,340, 1,310, 1,227, 1,159, 1,099, 1,031, 986, 769, 695 cm^{-1}; HRMS (EI^+): *m/z*: calcd for $C_{14}H_{10}O_4$: 242.0579, Found 242.0579.

Biological activity

Antioxidant screening

DPPH radical scavenging activity Methanolic solutions of DPPH exhibit a strong purple color and strong absorption at 517 nm, and this is reduced in the presence of an antioxidant (Cefarelli *et al.*, 2006; Burits and Bucar, 2000). Thus, there is an inverse relationship between the concentration of remaining DPPH and antiradical activity. Various concentrations of the test compounds (25 to 100 µg/mL) in methanol (total 1 mL) were added to 4 mL of a 0.004 % (w/v) methanolic solution of DPPH. After a 30-min incubation period at room temperature, absorbance was read against a blank at 517 nm. Percentage free radical scavenging (%) was calculated using the following equation.

$$\text{Scavenging}\,\% = \left[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of DPPH solution (containing all reagents except the test compound), and A_{sample} is the absorbance of the tested sample. Tests were conducted in triplicate.

Nitric oxide (NO) scavenging activity Nitric oxide scavenging activity was measured as described by Marcocci *et al.*, (1994) with slight modification. NO radicals were generated from sodium nitroprusside. Sodium nitroprusside (10 mM, 1 mL) and 1.5 mL of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations

(25 to 100 μg/mL) of the test compounds (**3** and **11**–**22**) and incubated for 150 min at 25°C. After incubation, 1 mL of the reaction mixture was treated with 1 mL of Griess reagent (1 % sulfanilamide, 2 % H_3PO_4 and 0.1 % naphthylethylenediaminedihydrochloride). Chromatophore absorbance was measured at 546 nm. Nitric oxide scavenging activity was calculated using the following equation.

$$\%\ \text{of scavenging} = \left[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were conducted in triplicate.

Hydrogen peroxide (H_2O_2) scavenging activity The H_2O_2 scavenging ability was determined as described by Ruch *et al.* (1989). In brief, a solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4), and then test compounds at 25 to 100 μg/mL in 3.4 mL of phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). The absorbance values of reaction mixtures were recorded at 230 nm. H_2O_2 scavenging percentages were calculated using the following equation.

$$\%\ \text{of scavenging} = \left[(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compounds. Tests were conducted in triplicate. The antioxidant activity of each sample was also expressed in terms of IC_{50} (microgram concentration required to inhibit the DPPH radical formation by 50 %) and was calculated from the graph after plotting inhibition percentage against log concentration.

Measurement of antibacterial activity using the disk diffusion method

Antibacterial activity of the tested samples was determined using a modified Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). In brief, 100 mL of the test bacteria was grown in 10 mL of fresh media until they reached a count of approximately 106 cells/mL for bacteria (Pfaller *et al.*, 1988). One hundred microliters of the bacterial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested. Of the many media available, the National Committee for Clinical Laboratory Standards (NCCLS) recommends Mueller–Hinton agar because it results in good batch-to-batch reproducibility (NCCLS, 1997). Gram-negative *E. coli* (KCTC-1924) and gram-positive *S. aureus* (KCTC-1916), both obtained from the Korean Collection for Type Cultures (KCTC), were incubated at 35–37 °C for 24–48 h, and then the diameters of the inhibition zones were measured in millimeters (Bauer *et al.*, 1966).

Standard disks of Ampicillin (antibacterial agent) served as positive controls for antibacterial activity, but filter disks impregnated with DMSO were used as a negative control. The agar used was Mueller–Hinton agar that is rigorously tested for composition and pH. Further, the depth of the agar in the plate is a factor to be considered in the disk diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values.

Blank paper disks with a diameter of 8.0 mm were impregnated with 10 mL of tested concentration of the stock solutions (100 μg/mL in DMSO). When a filter paper disk impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disk into the agar. This diffusion will place the chemical in the agar only around the disk. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disk. If an organism is placed on the agar, it will not grow in the area around the disk if it is susceptible to the chemical. This area of no growth around the disk is known as zone of inhibition or clear zone.

Determination of the minimal inhibitory concentration (MIC) Testing compounds and standard drug Ampicillin were well dissolved in DMSO at the concentration of 64 μg/mL. Then, twofold dilutions of the solution were prepared (64, 32…., 0.5 μg/mL). The microorganism suspensions at 106 CFU/mL (colony forming unit/mL) concentrations were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 h. The MIC was noted by observing the lowest concentration of the drug at which there was no visible growth.

Acknowledgments This research was supported by the Nano Material Technology Development Program through the Korean National Research Foundation (NRF) funded by the Korean Ministry of Education, Science, and Technology (2012M3A7B4049675).

References

Barclay LRC, Vinqvist MR, Mukai K, Itoh S, Morimoto H (1993) Chain-breaking phenolic antioxidants: steric and electronic effects in polyalkylchromanols, tocopherol analogs, hydroquinones, and superior antioxidants of the polyalkylbenzochromanol and naphthofuran class. J Org Chem 58:7416–7420. doi:10.1021/jo00078a020

Barclay LRC, Edwards CD, Mukai K, Egawa Y, Nishi T (1995) Chain-breaking naphtholic antioxidants: antioxidant activities of a polyalkylbenzochromanol, a polyalkylbenzochromenol, and 2,3-dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan compared to an α-tocopherol model in sodium dodecyl sulfate micelles. J Org Chem 60:2739–2744. doi:10.1021/jo00114a022

Barclay LRC, Antunes F, Egawa Y, McAllister KL, Mukai K, Nishi T, Vinqvist MR (1997) The efficiency of antioxidants delivered by liposomal transfer. Biochim Biophys Acta Biomembr 1328:1–12. doi:10.1016/s0005-2736(97)00057-6

Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496

Brimble MA, Burgess C, Halim R, Petersson M, Ray J (2004) Synthesis of pyrrolo[3,2-*b*]benzofurans and pyrrolo[3,2-*b*]naphthofurans via addition of a silyloxypyrrole to activated quinones. Tetrahedron 60:5751–5758. doi:10.1016/j.tet.2004.05.008

Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14:323–328. doi:10.1002/1099-1573(200008)14:5<323:aid-ptr621>3.0.co;2-q

Burton GW, Doba T, Gabe E, Hughes L, Lee FL, Prasad L, Ingold KU (1985) Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. J Am Chem Soc 107:7053–7065. doi:10.1021/ja00310a049

Cefarelli G, D'Abrosca B, Fiorentino A, Izzo A, Mastellone C, Pacifico S, Piscopo V (2006) Free-radical-scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. J Agric Food Chem 54:803–809. doi:10.1021/jf052632g

Chandrashekhar CH, Latha KP, Vagdevi HM, Vaidya VP (2011) Synthesis and antimicrobial activity of chalcones of naphtho[2,1-*b*]furan condensed with barbituric acid. Der Pharm Chem 3:365–369

Claessens S, Kesteleyn B, Van Nguyen T, De Kimpe N (2006) Synthesis of mollugin. Tetrahedron 62(35):8419–8424. doi:10.1016/j.tet.2006.06.014

Debnath AK, Hansch C, Kim KH, Martin YC (1993) Mechanistic interpretation of the genotoxicity of nitrofurans (antibacterial agents) using quantitative structure-activity relationships and comparative molecular field analysis. J Med Chem 36:1007–1016. doi:10.1021/jm00060a008

Einhorn J, Demerseman P, Royer R, Cavier R, Gayral P (1984) Effect of a substituent in the β position of the heterocycle on the antibacterial and parasiticidal properties of 2-nitronaphtho[2.1-*b*]furans. Eur J Med Chem Chim Ther 19:405–410

Engler TA, LaTessa KO, Iyengar R, Chai W, Agrios K (1996) Stereoselective syntheses of substituted pterocarpans with anti-HIV activity, and 5-aza-/5-thia-pterocarpan and 2-aryl-2,3-dihydrobenzofuran analogs. Bioorg Med Chem 4:1755–1769. doi:10.1016/0968-0896(96)00192-7

Gaggi R, Giovanninetti G, Garuti L (1982) β-Adrenolytic activity of some naphtho[2,1-*b*]furan derivatives. Farm Ed Sci 37:149–150

Giovanninetti G, Cavrini V, Chiarini A, Garuti L, Mannini-Palenzona A (1974) Substances with antiviral activity. I. Naphtho[2,1-*b*]furan-1(2*H*)-one, 1*H*-benz[*e*]indene-2,3-dihydro-1-one, and 1*H*-benz[*e*]indene-1,3(2H)-dione derivatives. Farm Ed Sci 29:375–385

Habonimana P, Claessens S, De Kimpe N (2006) Efficient syntheses of mollugin. Synlett 2472–2475. doi:10.1055/s-2006-950441

Ho L-K, Don M-J, Chen H-C, Yeh S-F, Chen J-M (1996) Inhibition of hepatitis B surface antigen secretion on human hepatoma cells. Components from *Rubia cordifolia*. J Nat Prod 59:330–333. doi:10.1021/np960200h

Hranjec M, Grdisa M, Pavelic K, Boykin DW, Karminski-Zamola G (2003) Synthesis and antitumor evaluation of some new substituted amidino-benzimidazolyl-furyl-phenyl-acrylates and naphtho[2,1-*b*]furan-carboxylates. Farmaco 58:1319–1324. doi:10.1016/s0014-827x(03)00197-6

Inoue K, Shiobara Y, Nayeshiro H, Inouye H, Wilson G, Zenk MH (1984) Quinones and related compounds in higher plants. Part 19. Biosynthesis of anthraquinones and related compounds in *Galium mollugo* cell suspension cultures. Phytochemistry 23:307–311. doi:10.1016/s0031-9422(00)80323-4

Jacobs J, Claessens S, Mbala BM, Huygen K, De Kimpe N (2009) New and highly efficient synthesis of 3-substituted 1-hydroxybenz[*g*]isoquinoline-5,10-diones. Tetrahedron 65:1193–1199. doi:10.1016/j.tet.2008.11.070

Jang T-S, Zhang HY, Kim GJ, Kim DW, Min B-S, Kang W, Son KH, Na MK, Lee SH (2012) Bioassay-guided isolation of fatty acid synthase inhibitory diterpenoids from the roots of *Salvia miltiorrhiza* Bunge. Arch Pharm Res 35:481–486. doi:10.1007/s12272-012-0311-8

Kwiecien H, Smist M, Kowalewska M (2012) Recent development on the synthesis of benzo[*b*]- and naphtho[*b*]furans: a review. Curr Org Synth 9:529–560. doi:10.2174/157017912802651393

Le GR, Oger F, Lecorgne A, Dudasova Z, Chevance S, Bondon A, Barath P, Simonneaux G, Salbert G (2009) Identification of small molecule regulators of the nuclear receptor HNF4α based on naphthofuran scaffolds. Bioorg Med Chem 17:7021–7030. doi:10.1016/j.bmc.2009.07.079

Lee YR, Kim BS (2001) Efficient synthesis of cytotoxic furonaphthoquinone natural products. Synth Commun 31:381–386. doi:10.1081/scc-100000527

Lee YR, Byun MW, Kim BS (1998) Efficient one-step synthesis of 2-arylfurans by ceric ammonium nitrate (CAN)-mediated cycloaddition of 1,3-dicarbonyl compounds to alkynes. Bull Korean Chem Soc 19:1080–1083

Lee YR, Kim BS, Kim DH (2000) Ceric ammonium nitrate (CAN)-mediated oxidative cycloaddition of 1,3-dicarbonyls to conjugated compounds. Efficient synthesis of dihydrofurans, dihydrofurocoumarins, dihydrofuroquinolinones, dihydrofurophenalenones, and furonaphthoquinone natural products. Tetrahedron 56:8845–8853. doi:10.1016/s0040-4020(00)00839-5

Lee YR, Lee GJ, Kang KY (2002) Ceric ammonium nitrate(CAN)-mediated oxidative cycloaddition of 1,3-dicarbonyls to vinyl sulfides. Application to the synthesis of evodone and avicequinone-B. Bull Korean Chem Soc 23:1477–1480. doi:10.5012/bkcs.2002.23.10.1477

Lee YR, Kang KY, Lee GJ, Lee WK (2003) Efficient synthesis of dihydrofurans with sulfide groups by ceric(IV) ammonium nitrate-mediated oxidative cycloaddition of 1,3-dicarbonyl compounds to vinyl sulfides. Application to the synthesis of benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione and first total synthesis of millettocalyxins C and pongamol methyl ether. Synthesis 1977–1988. doi:10.1055/s-2003-41023

Lumb J-P, Choong KC, Trauner D (2008) *ortho*-Quinone Methides from *para*-quinones: total Synthesis of Rubioncolin B. J Am Chem Soc 130:9230–9231. doi:10.1021/ja803498r

Mahadevan KM, Vaidya VP (2003) Synthesis and pharmacological evaluation of some potent naphtho[2,1-*b*]furopyrazolyl, -oxadiazolyl, and -coumaryl derivatives. Indian J Pharm Sci 65:128–134

Mahadevan KM, Padmashali B, Vaidya VP (2001) Studies in naphthofurans: Part V—synthesis of 2-aryl-1,2,3,4-tetrahydropyrido(naphtho[2,1-*b*]furan)-4-ones and their biological activity. Indian J Heterocycl Chem 11:15–20

Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L (1994) The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun 201:748–755. doi:10.1006/bbrc.1994.1764

Matthews JM, Chen X, Cryan E, Hlasta DJ, Rybczynski PJ, Strauss K, Tang Y, Xu JZ, Yang M, Zhou L, Demarest KT (2007) Design and synthesis of indane-ureido-thioisobutyric acids: a novel class of PPARα agonists. Bioorg Med Chem Lett 17:6773–6778. doi:10.1016/j.bmcl.2007.10.041

Nair V, Menon RS, Vinod AU, Viji S (2002) A facile three-component reaction involving [4 + 1] cycloaddition leading to furan annulated heterocycles. Tetrahedron Lett 43:2293–2295. doi:10.1016/s0040-4039(02)00226-5

NCCLS (National Committee for Clinical Laboratory Standards) (1997) Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Wayne, PA

Otani T, Sugimoto Y, Aoyagi Y, Igarashi Y, Furumai T, Saito N, Yamada Y, Asao T, Oki T (2000) New Cdc25B tyrosine phosphatase inhibitors, nocardiones A and B, produced by Nocardia sp. TP-A0248: taxonomy, fermentation, isolation, structural elucidation and biological properties. J Antibiot 53:337–344. doi:10.7164/antibiotics.53.337

Pfaller MA, Burmeister L, Bartlett MS, Rinaldi MG (1988) Multicenter evaluation of four methods of yeast inoculum preparation. J Clin Microbiol 26:1437–1441

Qian X, Zhang Y, Ni C (1994) The spectrum and antibacterial activity of naphthofurans and furans. J East China Inst Chem Technol 20:336–341

Rontó G, Gróf P, Buisson J-P, Einhorn J, Demerseman P (1992) Genotoxic effectivity. Comparison of 36 nitrated furan and arenofuran derivatives on a quantitative scale. Statistical comparison of T7 and other short-term tests. Mutagenesis 7:243–249. doi:10.1093/mutage/7.4.243

Ruch RJ, Cheng S, Klaunig JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis (Lond) 10:1003–1008. doi:10.1093/carcin/10.6.1003

Sastry MNV, Claessens S, Habonimana P, De Kimpe N (2010) Synthesis of the natural products 3-hydroxymollugin and 3-methoxymollugin. J Org Chem 75:2274–2280. doi:10.1021/jo100024b

Schildknecht H, Straub F (1976) Structure and synthesis of furomollugin from rhizomes of *Galium mollugo* L. (Rubiaceae). Justus Liebigs Ann Chem 1976:1772–1776

Shashikala DK, Ramaiah M, Vanita GK, Veena K, Vaidya VP (2011) Synthesis and analgesic activity of triazolothiadiazoles and triazolothiadiazines encompassing 3-nitronaphtho[2,1-*b*]furan. J Chem Pharm Res 3:445–451

Son JK, Jung SJ, Jung JH, Fang Z, Lee CS, Seo CS, Moon DC, Min BS, Kim MR, Woo MH (2008) Anticancer constituents from the roots of *Rubia cordifolia* L. Chem Pharm Bull 56:213–216. doi:10.1248/cpb.56.213

Srivastava V, Negi AS, Kumar JK, Faridi U, Sisodia BS, Darokar MP, Luqman S, Khanuja SPS (2006) Synthesis of 1-(3′,4′,5′-trimethoxy)phenylnaphtho[2,1-*b*]furan as a novel anticancer agent. Bioorg Med Chem Lett 16:911–914. doi:10.1016/j.bmcl.2005.10.105

Velmurugan D, Selvi UM, Mythily U, Rao K, Rajarajeshwari R, Sangeetha (2012) Structure-based discovery of anti-viral compounds for hepatitis B & C, human immunodeficiency, and dengue viruses. Curr Bioinform 7:187–211. doi:10.2174/157489312800604462

Wu L, Wang S, Hua H, Li X, Zhu t, Miyase T, Ueno A (1991) 6-Methoxygeniposidic acid, and iridoid glycoside from *Rubia cordifolia*. Phytochemistry 30:1710–1711. doi:10.1016/0031-9422(91)84241-j

Xia L, Lee YR (2013) A novel and efficient synthesis of diverse dihydronaphtho[1,2-*b*]furans using the ceric ammonium nitrate-catalyzed formal [3 + 2] cycloaddition of 1,4-naphthoquinones to olefins and its application to furomollugin. Org Biomol Chem 11:6097–6107. doi:10.1039/c3ob40977e