Synthesis and biological evaluation of berberine derivatives bearing 4-aryl-1-piperazine moieties

Bhupendra Mistry*, Young-Soo Keum and Doo Hwan Kim

Organic Research Laboratory, Department of Bioresources and Food Sciences, College of Life and Environmental Sciences, Konkuk University, Seoul, South Korea

Piperazine moieties with disubstituted *N*-aryl groups are linked to the isoquinoline alkaloid, berberine, through a pentyloxy side chain, replacing its 9-methoxyl group. The nine synthesised compounds are screened for antioxidant potency, *in vitro* anticancer activities against Hela and Caski cervical cancer cell lines and for cytotoxicity towards Malin Darby canine kidney cell lines. Several compounds demonstrate significant antioxidant potency and most of the compounds exhibit equipotent, or better, anticancer activity when compared to berberine.

Keywords: berberine, piperazine, antioxidant, cervical cancer

The occurrence of diseases like diabetes, cirrhosis, cancer and cardiovascular defects is associated with the presence of free radicals,[1,2] termed as reactive oxygen species (ROS). An imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage towards lipids, protein and nucleic acids, is termed oxidative stress.[3]Antioxidants are molecules, natural or synthetic, capable of interfering with free radicals and avoiding their sequence responses before essential vital components are damaged.[4] Research suggests that oxidative stress and ROS have a direct correlation with cancer and hence discovering new molecules capable of targeting both ROS scavenging, and cancer cell inhibition are of immense interest.

Natural products have been the single most useful source of leads for the development of drugs. Berberine, an isoquinoline alkaloid isolated from the roots and control debris of the Berberis varieties, is commonly used as a conventional medicine for treating diarrhoea and gastrointestinal disorders.[5] It possesses a variety of pharmacological properties such as antimicrobial, antileukaemic, antiulcerous, anti-inflammatory,[6] and anti-diarrhoea activities.[7] It also acts as an an enzyme inhibitor,[8,9] and as a glucose-lowering,[10] cholesterol-lowering,[11] neuroprotective,[12] antidepressant,[13] and Alzheimers disease-ameliorating agent.[14] It has also been reported that berberine analogues exhibit activity on LDLR,[15] and cytotoxicity against Hela, SVKO3 and Hep-2 cells,[16] and anti-leishmaniasis activity.[17] Research on the anticancer action of berberine, in particular, has attratcted extensive interest and there have been significant results.[18-24] However, since berberine is poorly absorbed in the digestive system it had a weak inhibitory impact on cancer cell growth, decreasing its effectiveness as an anticancer drug.[25,26] Hence, discovery of its derivatives which show pharmacological potential as anticancer agents better than berberine itself is of key importance in the ongoing anticancer drug development program. Because piperazines have useful pharmacological applications as anticancer agents[27] and antioxidants,[28] and inspired by the wide range of useful activities possessed by berberine derivatives we report here the synthesis of some novel piperazine-based berberine analogues.

Results and discussion

Scheme 1 shows the synthesis of the titled berberine-piperazine derivatives **4a–i**. Berberrubine (**2**), a demethylated derivative of berberine and a key intermediate was prepared by heating berberine in a vacuum oven at 190 °C temperature and 20–30 mm Hg pressure in 85% yield using a lietarture method.[29] In the final steps, dibromopentane in dry acetonitrile was employed for the alkylation of the 9-hydroxy group to yield intermediate compound **3** which was coupled with various piperazine moieties by a nucleophilic substitution reaction in DMF for 6–8 h to produce **4a–i** in reasonable yields.[30]

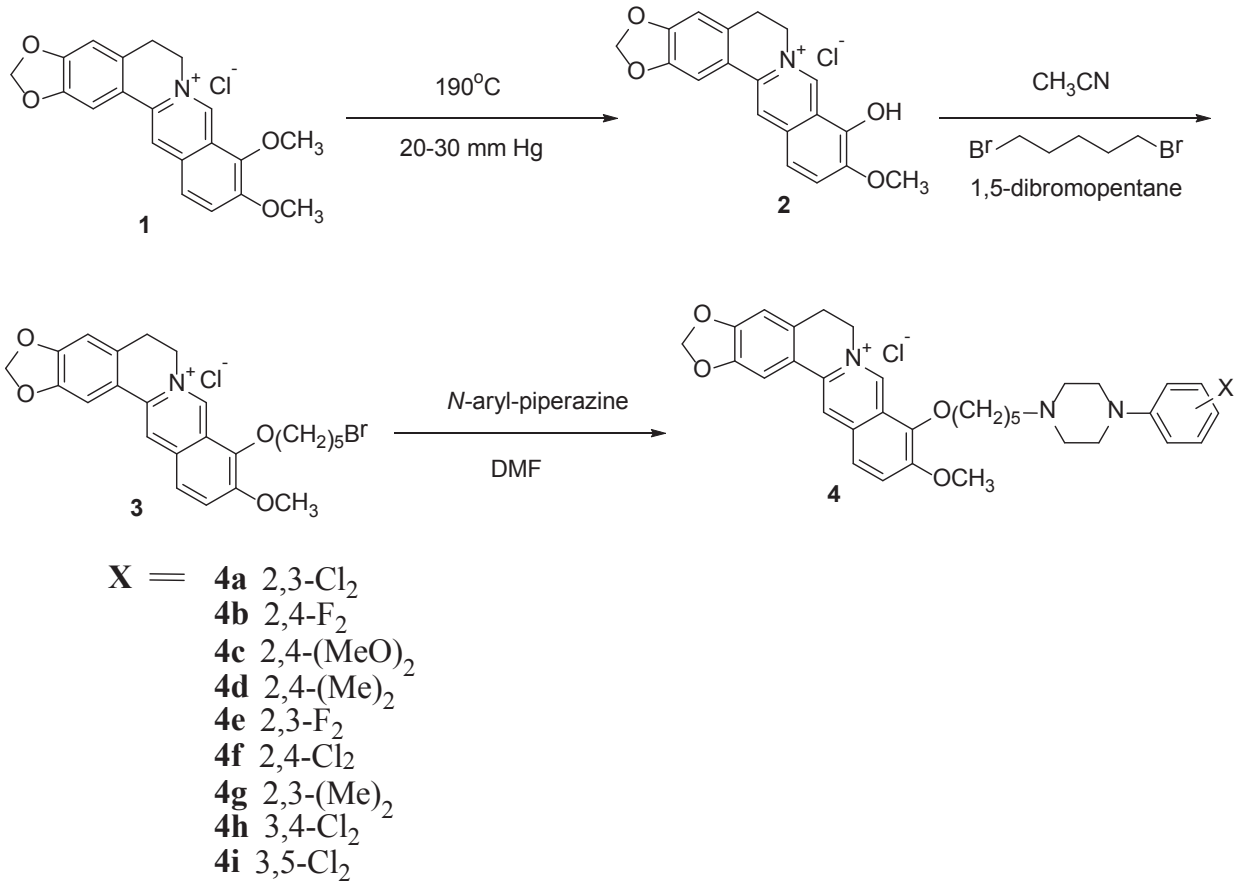
Spectroscopic techniques (IR, ^{1}H NMR, and mass spectrometry) and elemental analysis (CHN) were used to characterise compounds **4a–i.** The presence of C–H stretching for aromatics was confirmed on the basis of peaks observed around 3037–3061 cm^{-1}in the FTIR spectra of **4a–i**, and C=C stretching by bands at 1622–1531 cm^{-1}. The C–O–C band was seen as a sharp peak at 1121–1077 cm^{-1} along with , when appropriate, sharp chlorine bands at 757–781 cm^{-1}. The presence of the berberine ring was established on the basis of results of ^{1}H NMR spectra. For example, (**4b**) showed protons corresponding to H-8, H-13, H-1, H-12, H-4, H-11 as singlet peaks at 9.75, 8.66, 7.92, 7.51, 7.47, 6.77 ppm, respectively, as well as a singlet at 6.08 ppm due to the –OCH_2O of the berberine ring. The H-6 and H-5 methylate protons of the berberine ring appeared as triplets at 4.86 ppm and 2.55 ppm. A triplet for the alkyl chain protons (H-15) was observed at 4.41 ppm. A triplet signal at 3.25 ppm, a broad singlet at 2.41 ppm as well as multiplet signals at 2.24 ppm were due to the alkyl chain protons H-19, H-17 and H-18 and H-16, respectively. The presence of a methoxy functional group was confirmed by its characteristic peak at 4.05 ppm and the protons (H-23, H-25, H-20 and H-22) present on the piperazine moiety resonated as a broad singlet at around 3.79 ppm as well as at 3.45 ppm. The protons of the aromatic rings linked to the piperazine ring appeared as multiplets in the range 7.41–7.20 ppm. Mass spectrometric data (M+ ion values) for **4a–i** were in accordance with their structures.

Evaluation of biological activities

Antioxidant activities

The radical scavenging activity of berberine compounds **4a–i** towards radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) was measured with ascorbic acid as a standard compound. DPPH radical scavenging is considered a good *in vitro* model and is widely used to assess antioxidant efficacy conveniently.[31] Attachment of disubstituted piperazine derivatives to the berberine nucleus through a pentyl chain has an enhanced antioxidant effect as displayed in both the DPPH and ABTS bioassays. The activity level was found to vary according to

* Correspondent. E-mail: bhupendra.mistry84@gmail.com



X = **4a** 2,3-Cl_2
4b 2,4-F_2
4c 2,4-$(MeO)_2$
4d 2,4-$(Me)_2$
4e 2,3-F_2
4f 2,4-Cl_2
4g 2,3-$(Me)_2$
4h 3,4-Cl_2
4i 3,5-Cl_2

Scheme 1 Synthesis of piperazine linked berberine derivatives.

Table 1 Screening results for radical scavenging activity of berberine derivatives **4a–i** (Scheme 1)

Compound	IC_{50}[a]/μg mL^{-1} ± SD	
	DPPH	ABTS
4a	21.02 ± 0.82	6.501 ± 2.02
4b	24.40 ± 1.56	7.046 ± 3.11
4c	23.66 ± 1.83	7.522 ± 1.35
4d	17.90 ± 2.06	6.303 ± 0.74
4e	20.56 ± 0.52	7.681 ± 0.68
4f	18.63 ± 3.02	7.354 ± 1.26
4g	22.21 ± 1.24	7.852 ± 2.44
4h	11.08 ±0.47	4.768 ±0.66
4i	12.23 ± 068	4.876 ±0.48
Berberine	34.29 ± 1.73	82.17 ± 1.08
Ascorbic acid	10.75 ± 0.86	5.528 ± 0.88

[a]Antioxidant activities are shown as IC_{50} values in μg mL^{-1}. All assays were carried out in triplicate and the results expressed as average ± standard deviation.
ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

the type of functional groups present on the piperazine moiety. Overall, as can be seen from Table 1, most of the compounds showed significant antioxidant activity with IC_{50s} ranging from 11.08 to 24.40 μg mL^{-1} and 4.768 to 7.852 μg mL^{-1} in DPPH and ABTS bioassays, respectively. The highest scavenger activity observed in compounds **4h** and **4i** is probably due to the presence of dichloro functionality on piperazine rings system with IC_{50} levels of 11.08 μg mL^{-1} and 12.23 as well as 4.76 μg mL^{-1} and 4.876 μg mL^{-1}, respectively, in DPPH and ABTS bioassay. The, presence of electron donating dimethyl groups on the piperazine entity attached to the berberine ring was found to be beneficial for very good radical scavenging activity both in the DPPH assay (IC_{50} 17.90 μg mL^{-1}) and the ABTS assay (IC_{50} of 6.303 μg mL^{-1}). For the remaining analogues, variation in the activity level was observed in both the bioassays. For example an analogue **4a** with 2,3-dichloro substituents on the piperazine ring exhibited IC_{50}=6.501 μg mL^{-1} and **4f** with 2,4-dichloro sunstituents showed with IC_{50}=18.63 μg mL^{-1} in the ABTS and DPPH bioassays, respectively. Overall, the chlorine-based compounds emerged with significant radical scavenging efficacies, and their activity order falls as 3,4-Cl_2>3,5-Cl_2>2,3Cl_2>2,4-Cl_2. All the remaining compounds exhibited IC_{50} around 17–24 μg mL^{-1} in the DPPH assay as well as around 7 μg mL^{-1} in the ABTS assay which are considered to be good to moderate potencies when compared to the parent scaffold berberine with IC_{50}=34.29 μg mL^{-1} and 82.17 μg mL^{-1} and ascorbic acid with IC_{50}=10.75 μg mL^{-1} and 5.528 μg mL^{-1} in the DPPH and ABTS bioassays, respectively.

Anticancer activities

The anticancer potential of final berberine-based analogues **4a–i** was assayed against cervical cancer cell lines Hela and Caski, and the results of Sulforhodamine B colorimetric assay are presented in Tables 2 and 3. Overall, the compounds demonstrated low cytotoxicity towards the normal cell line, Malin Darby canine kidney (MDCK). But they showed excellent inhibitory effects against cervical cancer cell line Caski when compared to their potencies against Hela cell lines. Compounds **4a–i** demonstrated IC_{50}=5.697–6.807 μg mL^{-1} against Caski cell line as well as 5.923–7.849 μg mL^{-1} against the Hela cell line of cervical cancer. In addition, cytotoxicity levels were observed in the range 243.7–331.7 μg mL^{-1} and **4a–i** exhibited impressive

Table 2 Anticancer activity of synthesised compounds **4a–i** against HeLa and MDCK cancer cell lines and their toxicity

Compound	IC_{50}[a]/μg mL^{-1} ± SD HeLa	CC_{50}/μg mL^{-1} ± SD MDCK	TI
4a	5.578 ± 0.03	243.7 ± 0.88	43.69
4b	7.503 ± 0.07	331.7 ± 2.02	44.21
4c	7.849 ± 0.05	324.1 ± 2.34	41.29
4d	6.843 ± 0.02	306.1 ± 0.09	44.73
4e	7.576 ± 0.65	330.5 ± 1.53	43.62
4f	7.452 ± 1.07	308.3 ± 0.45	41.37
4g	7.237 ± 1.21	286.6 ± 0.78	39.60
4h	5.782 ± 0.55	320.7 ± 1.04	55.47
4i	5.923 ± 0.68	311.4 ± 0.63	52.57
Berberine	5.725 ± 0.01	161.2 ± 0.76	28.16

[a]Anticancer activities are shown as IC_{50} values in μg mL^{-1}. All assays were carried out in triplicate and the results expressed as average ± standard deviation.

CC_{50}, cytotoxicity concentration of 50%; MDCK, Malin Darby canine kidney; TI, therapeutic index.

Table 3 Anticancer activity of synthesised compounds **4a–i** against Caski and MDCK cancer cell lines and their toxicity

Compound	IC_{50}[a]/μg mL^{-1} ± SD Caski	CC_{50}/μg mL^{-1} ± SD MDCK	TI
4a	5.697 ± 0.37	243.7 ± 0.88	42.78
4b	6.133 ± 1.05	331.7 ± 2.02	54.08
4c	6.075 ± 1.58	324.1 ± 2.34	53.35
4d	6.405 ± 1.32	306.1 ± 0.09	47.79
4e	6.807 ± 2.06	330.5 ± 1.53	48.55
4f	6.412 ± 2.41	308.3 ± 0.45	48.08
4g	6.078 ± 0.92	286.6 ± 0.78	47.15
4h	5.634 ± 0.53	320.7 ± 1.04	56.92
4i	5.846 ± 0.48	311.4 ± 0.63	53.27
Berberine	5.983 ± 0.72	161.2 ± 0.76	26.94

[a]Anticancer activities are shown as IC_{50} values in μg mL^{-1}. All assays were carried out in triplicate and the results expressed as average ± standard deviation.

CC_{50}, cytotoxicity concentration of 50%; MDCK, Malin Darby canine kidney; TI, therapeutic index.

levels of therapeutic index ranging from 42.78 to 56.92 for Caski cell lines as well as 39.60 to 55.47 for Hela cell lines. Compound **4h** with a 3,4-dichlorophenyl piperazine entity was found to be the most active analogue among all those tested with IC_{50}=5.782 ± 0.55 μg mL^{-1} and CC_{50}=320.7 ± 1.04 μg mL^{-1} and the highest TI of 55.47 against Hela cell lines. The potency of this compound was double that of the parent berberine which showed a TI of 28.16. **4h** aslo has the most active effects against Caski cell lines with IC_{50}=5.634 ± 0.53 μg mL^{-1} and CC_{50}= 320.7 ± 1.04 μg mL^{-1} and a TI of 56.92, again more than double that of berberine with TI=26.94. The dichloro functionality was again found to be beneficial by contributing significant anticancer potential, as compound **4i** with a 3,5-dichlorophenyl piperazine entity appearing with IC_{50}=5.923 ± 0.68 μg mL^{-1} and 5.846 ± 0.48 μg mL^{-1} against Hela and Caski cell lines, respectively, as well as CC_{50}=311.4 ± 0.63 and TI=52.57 and 53.87, respectively. Compound (**4b**) with strong electronegative 2,4-difluoro substituents demonstrated IC_{50}=6.133 ± 1.05 μg mL^{-1} and CC_{50}=331.7 ± 2.02 μg mL^{-1} and TI= 54.08 against the Caski cell line. Compound **4c** with electron-donating alkoxy functionality exerted TI=53.35, the same as **4i** against Caski cell lines. Hence, the results clearly indicated that the berberine-piperazine adducts were more active against Caski cell lines than Hela. Overall, concerning the functionality, the anticancer activity order falls as Cl>F>OCH_3>CH_3. All the compounds exhibited similar double anticancer potentials against both the cervical cancer cell lines than did the parent berberine.

Conclusion

In summary, most of the synthesised compounds were potential leads for antioxidant activity. On the basis of observed results, it may be concluded that disubstitution groups favours activity. The chloro and methyl disubstitution increases the DPPH as well as ABTS free radical scavenging activity as compared to the potency of the parent berberine. All analogues bearing dichloro substituents showed a significant level of radical scavenging efficacy, particularly the 3,4-dichloroa and 3,5-dichloro compounds. All compounds exhibited antioxidant activities (IC_{50}=4.768–7.852 μg mL^{-1}) equipotent to the control ascorbic acid at IC_{50}=5.528 μg mL^{-1}, and hence can be considered further for development as antioxidant drugs. In addition, the dichloro compounds showed excellent anticancer effects against cervical cancer cell lines Hela and Caski, proving the importance of dichloro substitution on the piperazine rings system with around IC_{50}=5–6 μg mL^{-1} and TI level of >50 against both cell lines when compared to that of berberine against Hela and Caski, respectively. Overall, all compounds gave enhanced antioxidant and anticancer effects compared to the parent berberine, and equipotent potencies when compared to the control drugs..

Experimental

Highest quality chemicals and reagents (Sigma) were used in this study without prior purification. Veego Open capillary electronic apparatus VMP-D was utilized to obtain melting points which were uncorrected. A Shimadzu 8400-S FTIR spectrophotometer (KBr pellets) were used to obtain IR spectra. ^{1}H NMR spectra were obtained on a Varian 400 spectrometer in $CDCl_3$ using TMS as an internal reference. Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. TLC was carried out using appropriate mobile phase systems and silica gel-G coated microscopic glass slides (2x7.5 cm), and TLC spots were observed in a UV light chamber. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyser.

Berberrubine (**2**): Berberine hydrochloride (10 g, 0.01 mol) was added to a 50 mL round bottomed flask. The mixture was kept under reduced pressure (20–30 mmHg) and heated to 190° C for 40 min. The vacuum pump was turned off after the temperature decreased to room temperature. The reaction product was purified via silica gel column chromatography ($CHCl_3$/CH_3OH:15:1 and 10:1, eluting until no compound was observed in the eluent) to yield **2** asa brownish red amorphous powder (6.6 g, 85%).[29]

Bromopentylberberrubine (**3**): A solution of 2 (5 g, 0.01 mol)l and 1,5-dibromopentane (0.01 mol) in dry acetonitrile was heated at reflux for 6 h and then diethyl ether was added. The resulting solid was filtered off and then subjected to anion-exchange to convert into its chloride form **3**. Yield 61%; m.p. 195–197°C (DMF); IR (KBr) cm^{-1}: 3065 (C–H, Ar), 1615–1565 (C=C, Ar), 1115–1050 (C–O–C); ^{1}H NMR: δ 9.86 (s, 1H, H-8), 8.49 (s,1H, H-13), 7.86 (s, 1H, H-1), 7.54 (s, 1H, H-12), 7.54 (s, 1H, H-4), 6.84 (s, 1H, H-11), 6.03 (s, 2H, –OCH_2O), 4.93 (t, 2H, *J* = 6.3, H-6), 4.35 (t, 2H, *J* = 6.5, H-15), 4.03 (s, 3H, –OCH_3), 3.19 (t, 2H, *J* = 6.4, H-19), 2.58 (t, 2H, *J* = 7.4, H-5), 2.43 (br s, 4H, H-17, H-18), 2.21 (m, 2H, H-16); EMI–MS (*m/z*): 507.73 (M^+); Anal. calcd for $C_{24}H_{25}BrClNO_4$: C, 56.88; H, 4.97; N, 2.76; found: C, 56.75; H, 5.07; N, 2.54%.

*Synthesis of derivatives (***4a–i***); general procedure*

The substituted piperazine (0.01 mol) was added to a magnetically stirred solution of compound **3** and anhydrous K_2CO_3 in dry DMF (25 mL). The reaction mixture was heated at 80°C for 6-8 h and the reaction was monitored by TLC. The resulting solid was filtered off at room temperature and subjected to anion exchange to convert into its chloride form. The crude product was chromatographed on an Al_2O_3 column, eluted with $CHCl_3$/CH_3OH (9:1, v/v) to give the proposed compound.

9-O-3-(1-(2 3-Dichlorophenyl)piperazine)pentylberberine **(4a)**: Light yellow solid; yield 61%; m.p. 217–219°C (DMF); IR (KBr) cm^{-1}: 3052 (C–H, Ar), 1608–1559 (C=C, Ar), 1103–1080 (C–O–C), 781 (C–Cl); ^{1}H NMR: δ 9.82 (s, 1H, H-8), 8.41 (s,1H, H-13), 7.81 (s, 1H, H-1), 7.59 (s, 1H, H-12), 7.51 (s, 1H, H-4), 7.39–7.17 (m, 3H, ArH, piperazine H-27, H-28, H-29), 6.81 (s, 1H, H-11), 6.11 (s, 2H, $-OCH_2O$), 4.91 (t, 2H, *J* = 6.3, H-6), 4.38 (t, 2H, *J* = 6.5, H-15), 4.11 (s, 3H, $-OCH_3$), 3.89 (br s, 4H, piperazine, H-23, H-25), 3.51 (br s, 4H, piperazine, H-20, H-22), 3.21 (t, 2H, *J* = 6.4, H-19), 2.61 (t, 2H, *J* = 7.4, H-5), 2.49 (br s, 4H, H-17, H-18), 2.16 (m, 2H, H-16); EMI–MS (*m/z*): 656.37 (M^+). Anal. calcd for $C_{34}H_{36}Cl_3N_3O_4$: C, 62.15; H, 5.52; N, 6.40; found: C, 62.27; H, 5.43; N, 6.53%.

9-O-3-(1-(2,4-Difluorophenyl)piperazine)pentylberberine **(4b)**: Light yellow solid; yield 49%; m.p. 219–221°C (DMF); IR (KBr) cm^{-1}: 3048 (C–H, Ar), 1611–1547 (C=C, Ar), 1112–1085 (C–O–C); ^{1}H NMR: δ 9.75 (s, 1H, H-8), 8.66 (s,1H, H-13), 7.92 (s, 1H, H-1), 7.51 (s, 1H, H-12), 7.47 (s, 1H, H-4), 7.41–7.20 (m, 3H, ArH, piperazine H-27, H-28, H-30), 6.77 (s, 1H, H-11), 6.08 (s, 2H, $-OCH_2O$), 4.86 (t, 2H, *J* = 6.5, H-6), 4.41 (t, 2H, *J* = 6.6, H-15), 4.05 (s, 3H, OCH_3), 3.79 (br s, 4H, piperazine, H-23, H-25), 3.45 (br s, 4H, piperazine, H-20, H-22), 3.25 (t, 2H, *J* = 6.5, H-19), 2.55 (t, 2H, *J* = 7.5, H-5), 2.41 (br s, 4H, H-17, H-18), 2.24(m, 2H, H-16); EMI–MS (*m/z*): 624.52 (M^+). Anal. calcd for $C_{34}H_{36}ClF_2N_3O_4$: C, 65.43; H, 5.81; N, 6.73; found: C, 65.59; H, 5.93; N, 6.58%.

9-O-3-(1-(2,4-Dimethoxyphenyl)piperazine)pentylberberine **(4c)**: Light yellow solid; yield 47%; m.p. 239–241°C (DMF); IR (KBr) cm^{-1}: 3061 (C–H, Ar), 1606–1531 (C=C, Ar), 1121–1088 (C–O–C); ^{1}H NMR: δ 9.67 (s, 1H, H-8), 8.54 (s,1H, H-13), 7.80 (s, 1H, H-1), 7.61 (s, 1H, H-12), 7.43 (s, 1H, H-4), 7.37–7.15 (m, 3H, ArH, piperazine H-27, H-28, H-30), 6.69 (s, 1H, H-11), 6.15 (s, 2H, $-OCH_2O$), 4.95 (t, 2H, *J* = 6.4, H-6), 4.35 (t, 2H, *J* = 6.5, H-15), 4.21 (s, 3H, OCH_3), 4.03 (s, 3H, Ar–OCH_3 of piperazine), 3.95 (s, 3H, Ar–OCH_3 of piperazine), 3.83 (br s, 4H, piperazine, H-23, H-25), 3.41 (br s, 4H, piperazine, H-20, H-22), 3.18 (t, 2H, *J* = 6.3, H-19), 2.49 (t, 2H, *J* = 7.3, H-5), 2.47 (br s, 4H, H-17, H-18), 2.17 (m, 2H, H-16); EMI–MS (*m/z*): 648.06 (M^+). Anal. calcd for $C_{36}H_{42}ClN_3O_6$: C, 66.71; H, 6.53; N, 6.48; found: C, 66.63; H, 6.68; N, 6.61%.

9-O-3-(1-(2,4-Dimethylphenyl)piperazine)pentylberberine **(4d)**: Light yellow solid; yield 55%; m.p. 227–229°C (DMF); IR (KBr) cm^{-1}: 3053 (C–H, Ar), 1617–1567 (C=C, Ar), 1107–1079 (C–O–C); ^{1}H NMR: δ 9.86 (s, 1H, H-8), 8.61 (s,1H, H-13), 7.88 (s, 1H, H-1), 7.68 (s, 1H, H-12), 7.49 (s, 1H, H-4), 7.40–7.23 (m, 3H, ArH, piperazine H H-27, H-28, H-30), 6.75 (s, 1H, H-11), 6.05 (s, 2H, $-OCH_2O$), 4.88 (t, 2H, *J* = 6.4, H-6), 4.39 (t, 2H, *J* = 6.4, H-15), 4.13 (s, 3H, OCH_3), 3.78 (br s, 4H, piperazine, H-23, H-25), 3.53 (br s, 4H, piperazine, H-20, H-22), 3.22 (t, 2H, *J* = 6.5, H-19), 2.62 (t, 2H, *J* = 7.5, H-5), 2.43 (br s, 4H, H-17, H-18), 2.20 (m, 2H, H-16), 2.07 (s, 3H, Ar-CH3 of piperazine), 1.95 (s, 3H, Ar-CH3 of piperazine); EMI–MS (*m/z*): 616.44 (M^+). Anal. calcd for $C_{36}H_{42}ClN_3O_4$: C, 70.17; H, 6.87; Cl, 5.73; N, 6.82; found: C, 70.06; H, 6.74; N, 6.69%.

9-O-3-(1-(2 3-Difluorophenyl)piperazine)pentylberberine **(4e)**: Light yellow solid; yield 43; m.p. 211–213°C (DMF); IR (KBr) cm^{-1}: 3044 (C–H, Ar), 1622–1566 (C=C, Ar), 1102–1077 (C–O–C); ^{1}H NMR: δ 9.66 (s, 1H, H-8), 8.71 (s,1H, H-13), 7.95 (s, 1H, H-1), 7.57 (s, 1H, H-12), 7.41 (s, 1H, H-4), 7.32–7.11 (m, 3H, ArH, piperazine H-27, H-28, H-29), 6.73 (s, 1H, H-11), 6.13 (s, 2H, $-OCH_2O$), 4.78 (t, 2H, *J* = 6.3, H-6), 4.29 (t, 2H, *J* = 6.4, H-15), 4.07 (s, 3H, OCH_3), 3.85 (br s, 4H, piperazine, H-23, H-25), 3.46 (br s, 4H, piperazine, H- H-20, H-22), 3.11 (t, 2H, *J* = 6.3, H-19), 2.58 (t, 2H, *J* = 7.4, H-5), 2.39 (br s, 4H, H-17, H-18), 2.27 (m, 2H, H-16); EMI–MS (*m/z*): 624.09 (M^+). Anal. calcd for $C_{34}H_{36}ClF_2N_3O_4$: C, 65.43; H, 5.81; N, 6.73; found: C, 65.58; H, 5.68; N, 6.86%.

9-O-3-(1-(2,3-Dimethylphenyl)piperazine)pentylberberine **(4g)**: Light yellow solid; yield 57; m.p. 223–225°C (DMF); IR (KBr) cm^{-1}: 3041 (C–H, Ar), 1603–1558 (C=C, Ar), 1115–1076 (C–O–C); ^{1}H NMR: δ 9.77 (s, 1H, H-8), 8.51 (s,1H, H-13), 7.93 (s, 1H, H-1), 7.58 (s, 1H, H-12), 7.44 (s, 1H, H-4), 7.33–7.08 (m, 3H, ArH, piperazine H-27, H-28, H-29), 6.70 (s, 1H, H-11), 6.17 (s, 2H, $-OCH_2O$), 4.77 (t, 2H, *J* = 6.3, H-6), 4.43 (t, 2H, *J* = 6.5, H-15), 4.09 (s, 3H, OCH_3), 3.88 (br s, 4H, piperazine, H-23, H-25), 3.49 (br s, 4H, piperazine, H- H-20, H-22), 3.16 (t, 2H, *J* = 6.5, H-19), 2.48 (t, 2H, *J* = 7.4, H-5), 2.45 (br s, 4H, H-17, H-18), 2.23 (m, 2H, H-16), 2.11 (s, 3H, Ar-CH3 of piperazine), 1.90 (s, 3H, Ar-CH3 of piperazine); EMI–MS (*m/z*): 616.52 (M^+). Anal. calcd for $C_{36}H_{42}ClN_3O_4$: C, 70.17; H, 6.87; N, 6.82; found: C, 70.05; H, 6.73; N, 6.95%.

9-O-3-(1-(3,4-Dichlorophenyl)piperazine)pentylberberine **(4h)**: Light yellow solid; yield 62%; m.p. 259–261°C (DMF); IR (KBr) cm^{-1}: 3037 (C–H, Ar), 1615–1575 (C=C, Ar), 1106–1083 (C–O–C) 757 (C–Cl); ^{1}H NMR: δ 9.83 (s, 1H, H-8), 8.44 (s,1H, H-13), 7.85 (s, 1H, H-1), 7.66 (s, 1H, H-12), 7.53 (s, 1H, H-4), 7.34–7.13 (m, 3H, ArH, piperazine H-27, H-28, H-31), 6.83 (s, 1H, H-11), 6.07 (s, 2H, $-OCH_2O$), 4.79 (t, 2H, *J* = 6.5, H-6), 4.33 (t, 2H, *J* = 6.6, H-15), 4.12 (s, 3H, OCH_3), 3.77 (br s, 4H, piperazine, H-23, H-25), 3.43 (br s, 4H, piperazine, H-20, H-22), 3.15 (t, 2H, *J* = 6.3, H-19), 2.51 (t, 2H, *J* = 7.3, H-5), 2.35 (br s, 4H, H-17, H-18), 2.15 (m, 2H, H-16); EMI–MS (*m/z*): 656.37 (M^+). Anal. calcd for $C_{34}H_{36}Cl_3N_3O_4$: C, 62.15; H, 5.52; N, 6.40; found: C, 62.04; H, 5.42; N, 6.26%.

9-O-3-(1-(3 5-Dichlorophenyl)piperazine)pentylberberine **(4i):** Light yellow solid; yield 56; m.p. 245–247°C (DMF); IR (KBr) cm^{-1}: 3045 (C–H, Ar), 1601–1555 (C=C, Ar), 1111–1089 (C–O–C) 782 (C–Cl); ^{1}H NMR: δ 9.69 (s, 1H, H-8), 8.53 (s,1H, H-13), 7.90 (s, 1H, H-1), 7.55 (s, 1H, H-12), 7.46 (s, 1H, H-4), 7.35-7.19 (m, 3H, ArH, piperazine H-27, H-29, H-31), 6.78 (s, 1H, H-11), 6.09 (s, 2H, $-OCH_2O$), 4.83 (t, 2H, *J* = 6.4, H-6), 4.31 (t, 2H, *J* = 6.4, H-15), 4.19 (s, 3H, OCH_3), 3.90 (br s, 4H, piperazine, H-23, H-25), 3.47 (br s, 4H, piperazine, H-20, H-22), 3.24 (t, 2H, *J* = 6.3, H-19), 2.59 (t, 2H, *J* = 7.5, H-5), 2.33 (br s, 4H, H-17, H-18), 2.21 (m, 2H, H-16); EMI–MS (*m/z*): 656.07 (M^+). Anal. calcd for $C_{34}H_{36}Cl_3N_3O_4$: C, 62.15; H, 5.52; N, 6.40; found: C, 62.07; H, 5.66; N, 6.56%.

DPPH free radical scavenging assay

Free radical scavenging is one of the best-known mechanisms by which antioxidants inhibit lipid oxidation. DPPH and ABTS radical scavenging activity evaluations are standard assays in antioxidant activity studies. The antioxidant activity of the berberine derivatives **4a–i** was determined by these methods using ascorbic acid as standard.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the berberine derivatives **4a–i** were evaluated by adjusting the process of Kim *et al.*[32] Berberine derivatives (20 μL) were included in a 96-well microplate and 180 μL of DPPH was added to the wells. Methanol (20 μL) provided a blank and after 30 min incubation the optical density was determined at λ=517 nm. The control contains all reagents except the scavenger. The DPPH radical scavenging activity of ascorbic acid was also assayed for comparison; all tests were performed in triplicate. The results of this bioassay, RSA % (the radical scavenging activity in percentage) was determined as described in the following equation.

$$\%Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

A plot of concentration of test compounds and % scavenging introduced IC_{50} in the presence of ascorbic acid as standard.

ABTS radical cation decolourization assay protocol: 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Scavenging Assay. ABTS analysis was conducted by following the method of Lee *et al.*[33] In brief, 20 μL of sample was combined with 180 μL of ABTS radical solution followed by 10 min of incubation under dark conditions and the absorbance was measured at λ=734 nm. Ascorbic acid was used as a reference drug. The UV absorption data represented the radical scavenging rates which give the corresponding IC_{50s} for the test compounds.

The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$\%Scavenging = \frac{Absorbance\ of\ blank - Absorbance\ of\ test}{Absorbance\ of\ blank} \times 100$$

In vitro anticancer bioassay

Cell cultures: The cancerous cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 Medium (Sigma Aldrich Inc., USA) supplemented with 10% fetal bovine serum (FBS) and 1% of Antibiotic–Antimycotic solution (100x) were used for HeLa, Caski and MDCK cell growth respectively. DMEM, RPMI-1640, trypsin–EDTA, Antibiotic– Antimycotic Solution 100x and FBS were purchased from Welgene (150-Seongseo Industrial complex Bukro, Dalseogu, Daegu, 704–948 Republic of Korea). Three different kinds of cancerous cell lines, *viz*., HeLa (cervical), Caski (cervical) and MDCK (kidney) were plated in a 96-well plate at the density of 2×10^4 cells per well plate.

HeLa, Caski and MDCK cells analyses were performed by following the method of Pandurangan *et al*.[34]

This article was supported by the KU Research Professor Program of Konkuk University, Seoul, South Korea.

Received 28 June 2015; accepted 14 July 2015
Paper 1503449 doi: 10.3184/174751915X14381686689721
Published online: 7 August 2015

References

1 B. A. El-Gazzar, M.M. Youssef, A.M.S. Youssef, A.A. Abu-Hashem and F.A. Badria, *Eur. J. Med. Chem*., 2009, **44**, 609.
2 M.K. Kashif, I. Ahmad and S. Hameed, *Arkivoc*, 2008, **xvi**, 311.
3 G.C. Yen and H.Y. Chen, *Agric. Food Chem*., 1995, **43**, 27.
4 B.N. Ames, M.K. Shigenaga and T.M. Hagen, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 7915.
5 G. Waris and H. Ahsan, *J. Carcinog*., 2006, **5**, 14.
6 H.S. Bodiwala, S. Sabde, D. Mitra, K.K. Bhutani and I.P. Singh, *Eur. J. Med. Chem*., 2011, **46,** 1045.
7 C.L. Kuo, C.W. Chi and T.Y. Liu, *Cancer Lett*., 2004, **203**, 127.
8 L. Grycova, J. Dostal and R. Marek, *Phytochemistry*, 2007, **68**, 150.
9 R. Verpoorte, *Antimicrobially active alkaloids. Alkaloids: biochemistry, ecology, and medicinal applications.* Plenum Press, New York, 1998, pp. 397.
10 J. Yin, R. Hu, M. Chen, J. Tang, F. Li, Y. Yang and J. Chen, *Metabolism*., 2002, **51**, 1439.
11 S.H. Leng, F.E. Lu and L.J. Xu, *Acta Pharmacol. Sin*., 2004, **25,** 496.
12 W.H. Peng, K.L. Lo, Y.H. Lee, T.H. Hung and Y.C. Lin, *Life Sci*., 2007, **81**, 933.
13 H.S. Cui, K. Matsumoto, Y. Murakami, H. Hori, Q. Zhao and R. Obi, *Biol. Pharm. Bull.,* 2009, **32**, 79.
14 S.K. Kulkarni and A. Dhir, *Eur. J. Pharmacol*., 2007, **56**9, 77.
15 M. Asai, N. Iwata, A. Yoshikawa, Y. Aizaki, S. Ishiura, T.C. Saido and K. Maruyama, *Biochem. Biophys. Res. Commun*., 2007, **352**, 498.
16 P. Yang, D.Q. Song, Y.H. Li, W.J. Kong, Y.X. Wang, L.M. Gao, S.Y. Liu, R.Q. Cao and J.D. Jiang, *Bioorg. Med. Chem. Lett*., 2008, **18**, 4675.
17 L. Orfila, M. Rodriguez, T. Colman, M. Hasegawa, E. Merentes and F. Arvelo, *J. Ethnopharmacol*., 2000, **71**, 449.
18 J.I. Vennerstrom, J.K. Lovelace, V.B. Waits, W.I. Hanson and D.I. Klayman, *Agents Chemother*., 1990, **34**, 918.
19 A. Hoshi, T. Ikekawa, Y. Ikeda, S. Shirakawa and M. Iigo, *Gann*., 1976, **67**, 321.
20 C.Y. Lo, L.C. Hsu, M.S. Chen, Y.J. Lin, L.G. Chen, C.D. Kuo and J.Y. Wu, *Bioorg. Med. Chem. Lett*., 2013, **23**, 305.
21 J.Y. Pang, Y. Qin, W.H. Chen, G.A. Luo and Z.H. Jiang, *Bioorg. Med. Chem*., 2005, **13**, 5835.
22 Y. Ma, T.M. Ou, J.Q. Hou, Y.J. Lu, J.H. Tan, L.Q. Gu and Z.S. Huang, *Bioorg. Med. Chem*., 2008, **16**, 7582.
23 Q. Li, L. Zhang, Y. Zu, T. Liu, B. Zhang and W. He, *Biochem. Biophys. Res. Commun*., 2013, **433**, 432.
24 M. Franceschin, L. Rossetti, A.D. Ambrosio, S. Schirripa, A. Bianco, G. Ortaggi, M. Savino, C. Schultes and S. Neidle, *Bioorg. Med. Chem. Lett*., 2006, **16**, 1707.
25 E. Beausoleil, C. Chauvignac, T. Taverne, S. Lacombe, L. Pognante, B. Leblond, D. Pallares, C.D. Oliveira, F. Bachelot, R. Carton, H. Peillon, S. Coutadeur, V. Picard, N. Lambeng, L. Desire and F. Schweighofferm, *Bioorg. Med. Chem. Lett*., 2009, **19**, 5594.
26 G.Y. Pan, G.J. Wang, X.D. Liu, J.P. Fawcett and Y.Y. Xie, *Pharmacol. Toxicol*., 2002, **91**, 193.
27 C.M. Chen and H.C. Chang, *J. Chromatogr. B. Biomed. Appl*., 1995, **665**, 117.
28 R.V. Patel, P. Kumari, D.P. Rajani, C. Pannecouque, Erik De Clercq and K.H. Chikhalia, *Fut. Med. Chem*., 2012, **4**, 1053.
29 K. Iwasa, M. Kamigauchi, M. Ueki and M. Taniguchi, *Eur. J. Med. Chem*., 1996, **31**, 469.
30 W.J. Zhang, T.M. Ou, Y.J. Lu, Y.Y. Huang, W.B. Wu, Z.S. Huang, J.L. Zhou, K.Y. Wong and L.Q. Gu, *Bioorg. Med. Chem*., 2007, **15**, 5493.
31 K.H. Vijay, C.R. Gnanendra, N. Nagaraja and D.C. Gowda. *Eur. J. Chem*., 2008, **5**, 1123.
32 M.J. Kim, K.M. Maria John, J.N. Choi, S. Lee, A.J. Kim, Y. M. Kim and C.H. Lee. *Food Res. Int.,* 2013, **53**, 670.
33 S. Lee, S. Do, S.Y. Kim, J. Kim, Y. Jin, and C. H. Lee, *J. Agric. Food Chem*., 2012, **60**, 11222.
34 M. Pandurangan, G. Enkhtaivan, B. Mistry, M. Chandrasekran, R. Noorzai and D.H. Kim, *Saudi J. Biol. Sci*., 2015, http://dx.doi.org/10.1016/j.sjbs.2015.06.001.