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Design, synthesis and *in vitro* cytotoxicity studies of novel β -carbolinium bromides

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Abstract

A series of novel β -carbolinium bromides has been synthesized from easily accessible β -carbolines and 1-aryl-2-bromoethanones. The newly synthesized compounds were evaluated for their *in vitro* anticancer activity. Among the synthesized derivatives, compounds **16l**, **16o** and **16s** exhibited potent anticancer activity with IC₅₀ values of <10 μ M against tested cancer cell lines. The most potent analogue **16l** was broadly active against all the tested cancer cell lines (IC₅₀ = 3.16–7.93 μ M). In order to test the mechanism of cell death, we exposed castration resistant prostate cancer cell line (C4–2) to compounds **16l** and **16s**, which resulted in increased levels of cleaved PARP1 and AO/EB staining, indicating that β -carbolinium salts induce apoptosis in these cells. Additionally, the most potent β -carbolines **16l** and **16s** were found to inhibit tubulin polymerization.

Keywords

β -Carbolinium bromides; *In vitro* cytotoxicity; Microwave; Apoptosis

Introduction

β -Carboline unit containing natural products and synthetic molecules often exhibit a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, antioxidant, anticonvulsant, antitumor, antiviral, antiparasitic and antimicrobial.¹ Particularly, β -carboline analogues have been reported to exhibit significant antitumor activities against several human cancer cells (Fig. 1).^{2–4} For example, natural β -carbolines Harmine (**1**) and

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A. Supplementary material

Supplementary data (experimental procedures for the synthesis of compounds 10, 11, 12, 13, 15 and 16a–t, protocol for cytotoxicity assay and analytical spectra of final compounds 16a–t). Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2017.02.010>.

Fascaplysin (**2**) exhibited interesting antiproliferative activity through apoptosis induction, DNA intercalation and inhibition of Cyclin Dependent Kinases.^{5–10} Cao research group identified benzyl- β -carbolinium bromide **3** (IC_{50} = 0.8–8 μ M) and **4–5** (IC_{50} = 0.4–2.7 μ M) as potent cytotoxic agent by modification of natural Harmine.^{11,12} In 2012, Frederick and co-workers prepared the trisubstituted Harmine derivative **6**, with impressive anti-cancer activity (IC_{50} = 0.7 μ M; OE33).¹³

Among the various ways to enhance aqueous solubility, alkylation of azaheterocycles may lead to azolium salts with enhanced water solubility.^{14,15} In the recent past, many cationic nitrogen heterocycles have been emerged as potent antitumor agents.^{16,17} For example, Zhang group reported imidazoliumbromides as potent cytotoxic agents (IC_{50} < 5.0 μ M).¹⁸ Zeng et al. prepared 1-mesityl-3-(2-naphthoyl-methano)-1*H*-imidazo-lium bromide **7** (IC_{50} = 0.3–5 μ M) with interesting anticancer activity *via* arresting cell cycle at G1 phase and induced apoptosis in K562 cells.¹⁹ Recently, Xu group identified a series of novel 1-((indol-3-yl) methyl)-1*H*-imidazolium salts **8** (IC_{50} = 1.89 μ M; HL60) as apoptosis inducing potent anticancer agents.²⁰

In an effort to identify potent cytotoxic agents, recently we designed and prepared synthetic indolyl heterocycles with potent anticancer activities.^{21–23} Inspired by the fascinating anticancer properties of β -carboline and azolium salts, in this paper we designed a diverse series of β -carbolinium salts by incorporating remarkable features of β -carboline and 1-aryl-2-bromoethanones in single molecule as depicted in Fig. 2.

Substituted β -carboline intermediates **13** were prepared from L -tryptophan **9** as illustrated in Scheme 1.²⁴ Firstly, the reaction of **9** with formaldehyde solution (3.5 mL, 37%) under basic conditions produced tetrahydro β -carboline-3-carboxylic acid **10**. However, under similar reaction conditions substituted tetrahydro β -carbo-line-3-carboxylic acid **11** could not be prepared. Alternatively, carboxylic acid **11** was prepared from the reaction of **9** with aliphatic or aromatic aldehydes under acidic conditions. Next, iodobenzene diacetate-mediated decarboxylative aromatization of **10** or **11** led to β -carbolines **12** in good yields. Reaction of **12** with an appropriate alkyl halide and sodium hydride produced *N*-alkylated β -carbolines **13** in excellent yields. On the other hand, required 1-aryl-2-bromoethanones **15** were prepared from the reaction of aryloethanones **14** with *N*-bromo-succinimide (NBS) in acetonitrile using *p*-toluenesulfonic acid (PTSA) as a catalyst in good yields (Scheme 1).²⁵ Finally, the reaction of β -carboline **13** and 1-aryl-2-bromoethanone **15** was performed in refluxing ethanol. After refluxing the reaction contents for 20 h, we isolated the β -carboline salt **16** only in moderate yield (55%). In an attempt to enhance the reaction efficiency and product yield, we performed the alkylation of β -carboline **13** in focused microwave (MW). Reaction of **13** and 1-aryl-2-bromoethanone **15** in focused MW led to β -carbolinium bromide **16a** with improved yield and notable reduced time (from hour to min.). MW-assisted organic synthesis has received substantial attention in pharmaceutical industry due to dramatic savings of reaction time and higher product yields.^{26,27} Initially, we irradiated the reaction mixtures in MW oven at 50°C for 20 min and obtained **16a** in 60% yield. Notably, by increasing reaction temperature from 50°C to 80°C, β -carbolinium bromide salt **16a** was produced in 89% yield. The generality of identified reaction conditions was

demonstrated by synthesizing an array of β -carbolinium bromides **16a–t** in good to excellent yields (75–92%). 1-Aryl-2-bromoethanones with electron-donating (CH_3 and OCH_3) and electron-withdrawing (Br) groups smoothly delivered **16** in high yields.

Structures of all the synthesized β -carbolinium bromides **16a–t** were confirmed by IR, NMR (^1H & ^{13}C) and mass spectral data. In ^1H NMR spectrum, two characteristic singlets appeared at ~ 9.5 and ~ 6.6 ppm due to C-1 proton of β -carboline and CH_2 - of arylacyl moiety at N^2 , respectively. ^{13}C NMR of **16** showed a characteristic signal at ~ 190 ppm for the carbonyl carbon ($\text{C}=\text{O}$). A band at 1690 cm^{-1} in IR spectra of **16** indicated the presence of $\text{C}=\text{O}$ functional group. The purity of carbolinium bromides **16a–t** was found to be greater than 97% by HPLC analysis.

In vitro cytotoxicity of β -carbolinium bromides **16a–t** was evaluated against pancreatic (BxPC-3), cervical cancer (HeLa), castration-resistant prostate (C4-2), androgen-independent prostate (PC3), human embryonic kidney 293 (HEK293T) and breast carcinoma (MDA-MB-231) cells by MTT assay. Doxorubicin was used as the reference drug. Activity results in terms of IC_{50} values are summarized in Table 1. Structure–activity relationship study was carried out by varying substituents on β -carboline (R and R^{11}) and 1-aryl-2-bromoethanone (Ar) moieties. Compound **16a** without any substituent on β -carboline and arylacyl moieties was found to be moderately active against a panel of cancer cell lines ($\text{IC}_{50} = 21.6\text{--}74.9\text{ }\mu\text{M}$). Replacement of phenyl with a *p*-tolyl ring in arylacyl part at N^2 led to **16b** with slightly improved cytotoxicity ($\text{IC}_{50} = 18.4\text{--}31.9\text{ }\mu\text{M}$, **16a** vs **16b**). Similarly, *p*-methoxyphenyl analogue **16c**, slightly augmented the growth inhibitory potency when compared to **16a** and **16b** ($\text{IC}_{50} = 13.2\text{--}55.8\text{ }\mu\text{M}$). Dimethoxyphenyl (**16d**) and trimethoxyphenyl (**16e**) derivatives were found to be weakly cytotoxic against kidney cells ($\text{IC}_{50} = 67.8$ and $28.6\text{ }\mu\text{M}$; HEK293T) and inactive against other cell lines. β -Carbo-line **16f** with electron-withdrawing (*p*-bromophenyl) substituent displayed improved cytotoxicity ($\text{IC}_{50} = 18.8\text{--}44.1\text{ }\mu\text{M}$) when compared to compound **16a**. 2-Naphthyl analogue **16g** showed improved activity with IC_{50} values ranging $11.1\text{--}40.4\text{ }\mu\text{M}$ (**16g** vs **16a**). Replacement of an aryl group with heteroaryl (furyl and thienyl) moiety led to compounds **16h–i** with weak cytotoxicity or inactive against the tested cell lines except **16i** with moderate cytotoxicity towards kidney (HEK293T) cells ($\text{IC}_{50} = 18.2\text{ }\mu\text{M}$). *N*-Methylated derivatives **16j–k** showed moderate cytotoxicity ($\text{IC}_{50} = 14\text{--}65\text{ }\mu\text{M}$) towards tested cancer cell lines. *N*-(4-Chlorobenzyl) unit is reported to be beneficial for the potency of various indole-based anticancer lead molecules.^{28,29} In an efforts to improve anticancer activity of the β -carbolinium bromides, we prepared *N*-(4-chloro-benzyl) β -carbolinium bromides **16l–n** with significant enhanced cytotoxicity against the tested cancer cells compared to β -carboline with free *N*-H. Notably, compound **16l** found to be the most potent analogue of the series with broad cytotoxicity against all the tested cell lines (IC_{50} $3.2\text{--}7.9\text{ }\mu\text{M}$). Incorporation of methyl and *p*-methoxyphenyl substituents at position 1 (**16o–s**) of β -carboline further increased the growth inhibitory potency ($\text{IC}_{50} = 8.7\text{--}49.2\text{ }\mu\text{M}$) when compared to **16c**, **16e** and **16g** ($\text{IC}_{50} = 11.1\text{--}116.3\text{ }\mu\text{M}$). *N*-Benzylation of **16q** led to **16t** with moderate cytotoxicity ($\text{IC}_{50} = 12.6\text{--}94.1\text{ }\mu\text{M}$). Overall, most of the β -carbolinium bromides proved to be active against kidney cells (HEK293T, $\text{IC}_{50} = 3.7\text{--}67.8\text{ }\mu\text{M}$). Activity results suggest that substituents at positions 1, 2 and 9 of β -carboline and arylacyl part bearing 4-methoxyphenyl

and 2-naphthyl groups are beneficial for the anticancer activity. Notably, the most potent compound **16l** with moderate water solubility (86 µg/mL) was found to be 95% stable at pH 4.5 up to 24 h.

To determine the preliminary mechanism of action of β -carbolinium bromides, PARP1 cleavage assay for the active compounds **16l** and **16s** was performed. C4–2 Cells were treated with **16l** and **16s** for 48 h, and cleaved PARP1 levels was analyzed using immunoblotting. As shown in Fig. 3, exposure of C4–2 cells by either **16l** or **16s** enhanced the levels of PARP1 cleavage as indicating apoptotic induced cell death in C4–2 cells.

Furthermore, we also conducted acridine orange/ethidium bromide assay to investigate the mechanism of cell death. Acridine orange (AO) stains both live and dead cells, whereas ethidium bromide (EB) only stains dead cells.³⁰ Therefore, AO/EB staining is used to examine whether cell death occurred via apoptosis or necrosis. Effect of β -carbolinium salts on the morphological changes of C4–2 cells is illustrated in Fig. 4. Incubation of compounds **16s** (IC₅₀ = 8 µM) and **16l** with C4–2 cells (IC₅₀ = 7 µM) for 24 h resulted in typical nuclear fragmentation (red), whereas no visible changes in cell nucleus and cell membrane integrity was observed for the control cells. The results of AO/EB staining revealed that compounds **16l** and **16s** trigger apoptosis in C4–2 cells, thereby confirming the PARP cleavage data.

In the recent past, indole-based compounds have been reported for their significant anticancer activity through modulation of tubulin-heterodimer dynamics and binding at colchicine binding sites.³¹ In order to find the theoretical binding sites of novel β -carboline bromides, a docking study for the identified potent compounds **16l** and **16s** was performed by Molegro Virtual docker program³² according to reported high-resolution crystal structure of the tubulin-DAMA-colchicine (CN-2) complex (PDB ID: 1SA0).³³ Scoring functions and hydrogen bond formed with the surrounding amino acids predicted the binding affinities for **16l** and **16s** with MolDock Scores of –151.18 and –167.90, respectively. The docking poses of the molecule with the receptor are detailed in Fig. 5. Binding interactions for **16l** are strongly stabilized by two hydrogen bonds; first interaction between C=O and Asp251 with hydrogen bond distance of 3.0616 Å; the second one between oxygen of 4-methoxyphenyl group and Cys241 with the bond length of 2.6103 Å. Similarly, compound **16s** also showed hydrogen-bonding interactions between the oxygen of 1-(4-methoxyphenyl)- β -carboline and Cys241 with 3.207 Å distance of hydrogen bond. Apart from hydrogen bonding interactions, compounds **16l** and **16s** also strongly stabilized by steric interactions (red dotted lines) as illustrated in Fig. 5. Additionally, the hydrophobic interactions in the colchicine-binding domain of the tubulin for β -carbolines **16l** and **16s** are shown in Fig. 6.

To validate the theoretical molecular-docking hypothesis, we examined the tubulin polymerization activity for the identified two potent compounds **16l** and **16s** in a cell free system. β -Carbolinium bromides **16l** and **16s** were found to inhibit tubulin polymerization at 6 µM as shown in Fig. 7.

In summary, a series of β -carbolinium bromides **16a–t** were prepared from easily accessible β -carbolines and 1-aryl-2-bromoethanones using MW irradiation. *In vitro* anticancer activities of β -carbolinium bromides **16a–t** were evaluated against six-human tumor cell

lines. β -Carboline **16l** displayed most potent cytotoxicity against the tested cancer cell lines with IC₅₀ values ranging 3.16–7.93 μ M. The preliminary mechanism of action study revealed that compounds **16l** and **16s** induced apoptotic cell death by enhancing the level of cleaved PARP1 in C4–2 cells and exhibited their activity through the inhibition of tubulin polymerization. This class of compounds can be further exploited for obtaining highly potent cytotoxic compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Representative β -carboline-based anticancer agents.

Fig. 2.
Rational design for β -carbolinium bromides.

Fig. 3.
PARP1 cleavage (in C4-2 cells) induced by **16l** and **16s**.

Fig. 4.
Apoptosis inducing effects of **16l** and **16s** in C4-2 cells.

Fig. 5. Binding interactions of **16l** and **16s** in the colchicine-binding site of tubulin. Hydrogen bonds (green and blue dotted lines) and steric interactions (red dotted lines) are outlined.

Fig. 6.
Hydrophobicity effect of **16l** and **16s** in the binding pocket of colchicine.

Fig. 7.
Effect of compounds **16l** and **16s** on *in vitro* tubulin polymerization.

Scheme 1.

Reagents and conditions: (a) 37% formaldehyde solution, 0.4 N NaOH, 37°C, 3 days, CH₃COOH, rt, 2 days; (b) RCHO, CH₃COOH, 100°C, 12 h; (c) IBD, DMF, rt, 2 h; (d) R¹X, NaH, DMF, rt, 12 h. (e) NBS, CH₃CN, PTSA, reflux, 4–5 h; (f) **13**, EtOH, MW, 80°C, 20 min.

Table 1

In vitro cytotoxicity of β -carbolinium bromides **16a-t** against a panel of cancer cells (IC₅₀ in μ M).

Compd	R	R ¹	Ar	BxPC-3	HeLa	C4-2	PC-3	HEK293T	MDA-MB-231
16a	H	H	C ₆ H ₅	27.8 ± 2.7	37.7 ± 3.4	74.9 ± 5.5	59.6 ± 5.3	21.6 ± 2.8	37.2 ± 4.1
16b	H	H	4-CH ₃ C ₆ H ₄	19.9 ± 2.3	27.5 ± 3.1	31.9 ± 3.5	39.1 ± 3.1	18.4 ± 4.1	29.2 ± 2.2
16c	H	H	4-CH ₃ OC ₆ H ₄	>100	48.7 ± 2.9	45.7 ± 5.4	55.8 ± 4.9	13.2 ± 2.6	>100
16d	H	H	3,4-(CH ₃ O) ₂ C ₆ H ₃	>100	>100	>100	>100	67.8 ± 7.9	>100
16e	H	H	3,4,5-(CH ₃ O) ₃ C ₆ H ₂	>100	>100	>100	>100	28.6 ± 3.8	>100
16f	H	H	4-BrC ₆ H ₄	24.5 ± 3.9	44.5 ± 5.0	33.3 ± 2.9	43.2 ± 5.2	18.8 ± 3.1	44.1 ± 5.3
16g	H	H	2-Naphthyl	15.1 ± 2.4	35.2 ± 2.6	40.4 ± 3.9	35.8 ± 3.0	11.1 ± 2.2	25.1 ± 3.6
16h	H	H	2-Furyl	>100	>100	>100	>100	>100	>100
16i	H	H	2-Thienyl	38.8 ± 4.9	65.0 ± 4.6	>100	>100	18.2 ± 2.6	48.9 ± 5.8
16j	H	CH ₃	4-CH ₃ OC ₆ H ₄	18.0 ± 2.1	55.6 ± 5.4	37.9 ± 4.3	35.2 ± 4.1	14.2 ± 2.8	38.1 ± 2.9
16k	H	CH ₃	2-Naphthyl	64.9 ± 4.3	33 ± 3.2	25.4 ± 2.2	43.8 ± 3.9	17.6 ± 3.0	54.2 ± 5.4
16l	H	4-ClC ₆ H ₄ CH ₂	4-CH ₃ OC ₆ H ₄	6.3 ± 1.0	3.2 ± 0.9	7.4 ± 1.2	5.4 ± 0.8	3.8 ± 1.1	7.9 ± 1.1
16m	H	4-ClC ₆ H ₄ CH ₂	3,4,5-(CH ₃ O) ₃ C ₆ H ₂	35.3 ± 2.9	13.16 ± 2.5	41.0 ± 3.8	36.5 ± 3.4	11.5 ± 3.1	37.0 ± 4.0
16n	H	4-ClC ₆ H ₄ CH ₂	2-Naphthyl	36.9 ± 3.6	14.2 ± 2.2	11.6 ± 2.1	15.2 ± 2.7	10.6 ± 2.8	16.2 ± 1.8
16o	CH ₃	H	4-CH ₃ OC ₆ H ₄	26.6 ± 1.8	23.9 ± 2.9	28.1 ± 2.6	26.7 ± 2.0	9.5 ± 1.1	16.8 ± 3.1
16p	CH ₃	H	3,4,5-(CH ₃ O) ₃ C ₆ H ₂	30.9 ± 3.2	54 ± 6.2	49.2 ± 3.4	39.5 ± 3.3	18.9 ± 2.0	40.2 ± 5.2

Compd	R	R ¹	Ar	BxPC-3	HeLa	C4-2	PC-3	HEK293T	MDA-MB-231
16q	CH ₃	H	2-Naphthyl	12.3±2.0	13±2.6	17.7±1.9	19.5±2.1	17.7±2.8	14.1 ± 2.1
16r	4-CH ₃ OC ₆ H ₄	H	4-CH ₃ OC ₆ H ₄	20.0 ± 2.5	26.4 ± 1.4	22.1 ± 2.9	28.1 ± 3.4	17.8±2.0	22.4 ± 2.7
16s	4-CH ₃ OC ₆ H ₄	H	2-Naphthyl	12.2±1.6	15.5±2.0	8.7 ± 1.5	10.1 ± 1.1	11.6±1.9	14.1 ± 2.1
16t	CH ₃	C ₆ H ₄ CH ₂	2-Naphthyl	94.1 ± 6.4	37.6 ± 2.4	12.6±2.3	15.3±1.4	29.0 ± 4.2	44.1 ± 6.3
Doxorubicin	14.3	4.85	2.5	14.3±2.3	4.8 ± 1.2	2.5 ± 0.8			

The activity data represent mean IC₅₀ values of experiments conducted in triplicates.