Synthesis and Cytotoxic Activity of New β-Carboline Derivatives

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Abstract: On the basis of harmine and 1-methoxy-canthin-6-one chemical structures, a series of novel 1,4-disubstituted and 1,4,9-trisubstituted β-carbolines and tetracyclic derivatives were designed and synthesized. Cytotoxic activities of these compounds *in vitro* were investigated in a human tumor cell line panel. Almost all compounds demonstrated interesting cytotoxic activities in particular against prostate cancer cells PC-3 with IC_{50} in the low micromolar range. Compound **X** was found to be the most potent one with IC_{50} value of 8.0 μM; this suggests further studies with models of prostate cancer.

Keywords: β-carbolines, 1-methoxycanthin-6-one, prostate cancer.

INTRODUCTION

Natural products have historically and continually been investigated for promising new leads in pharmaceutical development. The β-carboline alkaloids, that possess a common tricyclic pyrido[3,4-b]indole ring structure [1,2] have attracted attention regarding several aspects of medicinal chemistry. They were originally isolated from *Peganum harmala* (Zygophillaceae, Syrian Rue), which is being used as a traditional herbal drug as an emmenagogue and abortifacient in the Middle East and North Africa [3]. In the Amazon basin plants containing β-carbolines were widely used as hallucinogenic drinks or snuffs. Besides, the extracts of the seeds of *Peganum harmala* have been traditionally used for hundreds of years to treat the alimentary tract cancers and malaria in Northwest China [4].

These compounds possess a wide diversity of important biochemical effects and pharmacological properties. Numerous previous reports investigated the effects of β-carboline alkaloids on the central nervous system (CNS), such as their affinity with benzodiazepine receptors (BZRs), 5-HT_{2A} and 5-HT_{2C} receptors [5-7]. Recent interest in these alkaloids has been focused on their potent antitumor activity. Several investigations [8-20] on the synthesis of a variety of β-carboline derivatives and the evaluation of their antitumor activities unraveled that β-carbolines demonstrated potent antitumor activities and the activity was correlated to both the planarity of the molecule and the presence of the ring substituents. Recently it is discovered that β-carboline derivatives may function their antitumor activity through multiple mechanisms, such as intercalating into DNA [21], inhibiting Topoisomerase I and II [22], CDK [23-24], and IKK (IkB kinase complex) [25].

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Harmine (Fig. (**1**)) is the most representative β-carboline alkaloid endowed with antitumor properties. It showed high cytotoxicity both *in vitro* against different human tumor cell lines and *in vivo* in mice bearing both Lewis lung cancer and Sarcoma 180. It exhibited remarkable DNA intercalation capacity and significant Topo I inhibition activity [14,17].

Tetracyclic systems in which another ring has been fused to the β-carboline nucleus have also shown promising antitumor activity. In particular, 1-methoxycanthin-6-one (Fig. (**1**)), a natural product isolated from the medicinal plant Ailanthus altissima, showed interesting antiproliferative properties, suppressing the growth of a panel of human tumor cell lines, including epiderimoid carcinoma of the nasopharynx (KB), ileocecal carcinoma (HCT-8), renal cancer (CAK-1), breast cancer (MCF-7) and melanoma (SK-MEL-2) [26].

As part of our ongoing efforts, we recently explored the effects of 1-methoxycanthin-6-one on apoptosis in human leukemia (Jurkat), thyroid carcinoma (ARO and NPA), and hepatocellular carcinoma (HuH7) cell lines.

We demonstrated that 1-methoxy-canthin-6-one induced apoptosis *via* a JNK-dependent mechanism. Furthermore, the compound synergized with human recombinant tumor necrosis factor (TNF)–related apoptosis-inducing ligand (hrTRAIL) in apoptosis induction [27].

Inspired by these results, in the present investigation, we designed and synthesized novel 1,4-disubstituted and 1,4,9-trisubstituted β-carbolines and 1-methoxycanthin-6-one derivatives (Fig. (**2**)). The purpose of this study is to evaluate the antiproliferative properties of these compounds in order to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties and antitumor activities.

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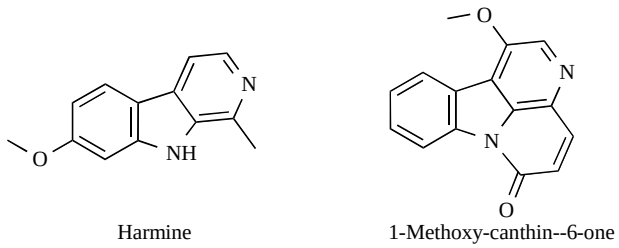


Fig. (1). Chemical structure of harmine and 1-methoxycanthin-6-one.

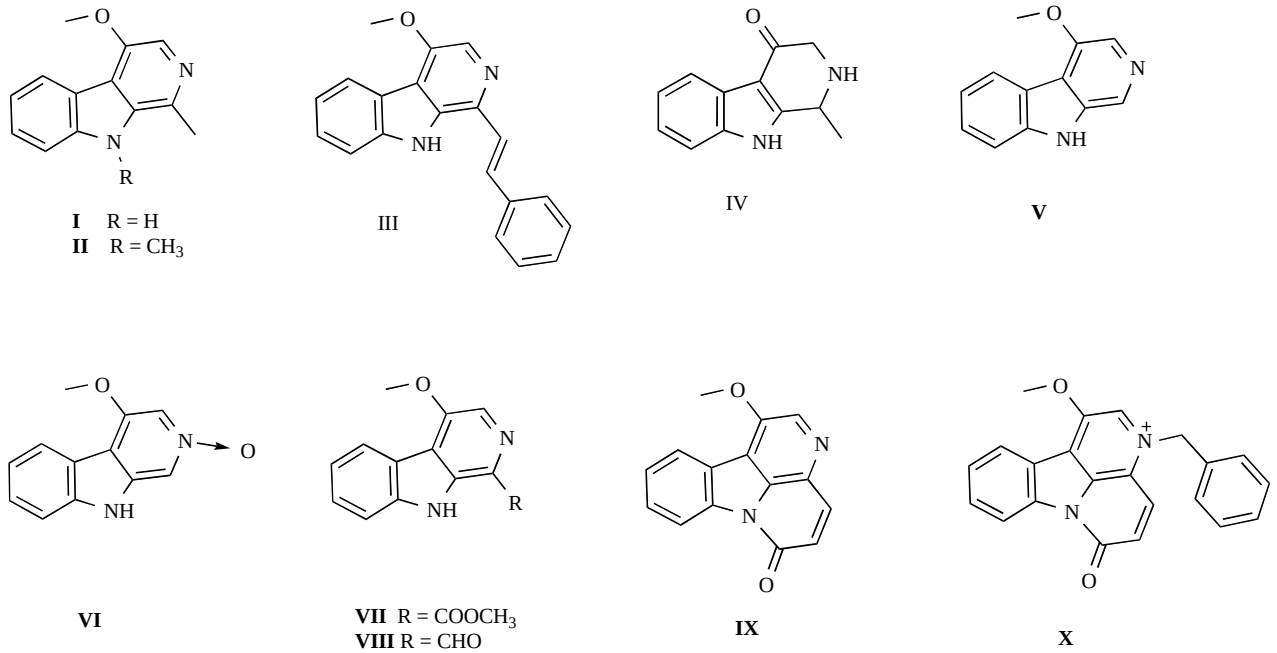


Fig. (2). Chemical structure of 1,4-disubstituted and 1,4,9-trisubstituted β-carbolines and tetracyclic derivatives.

RESULTS AND DISCUSSION

Chemistry

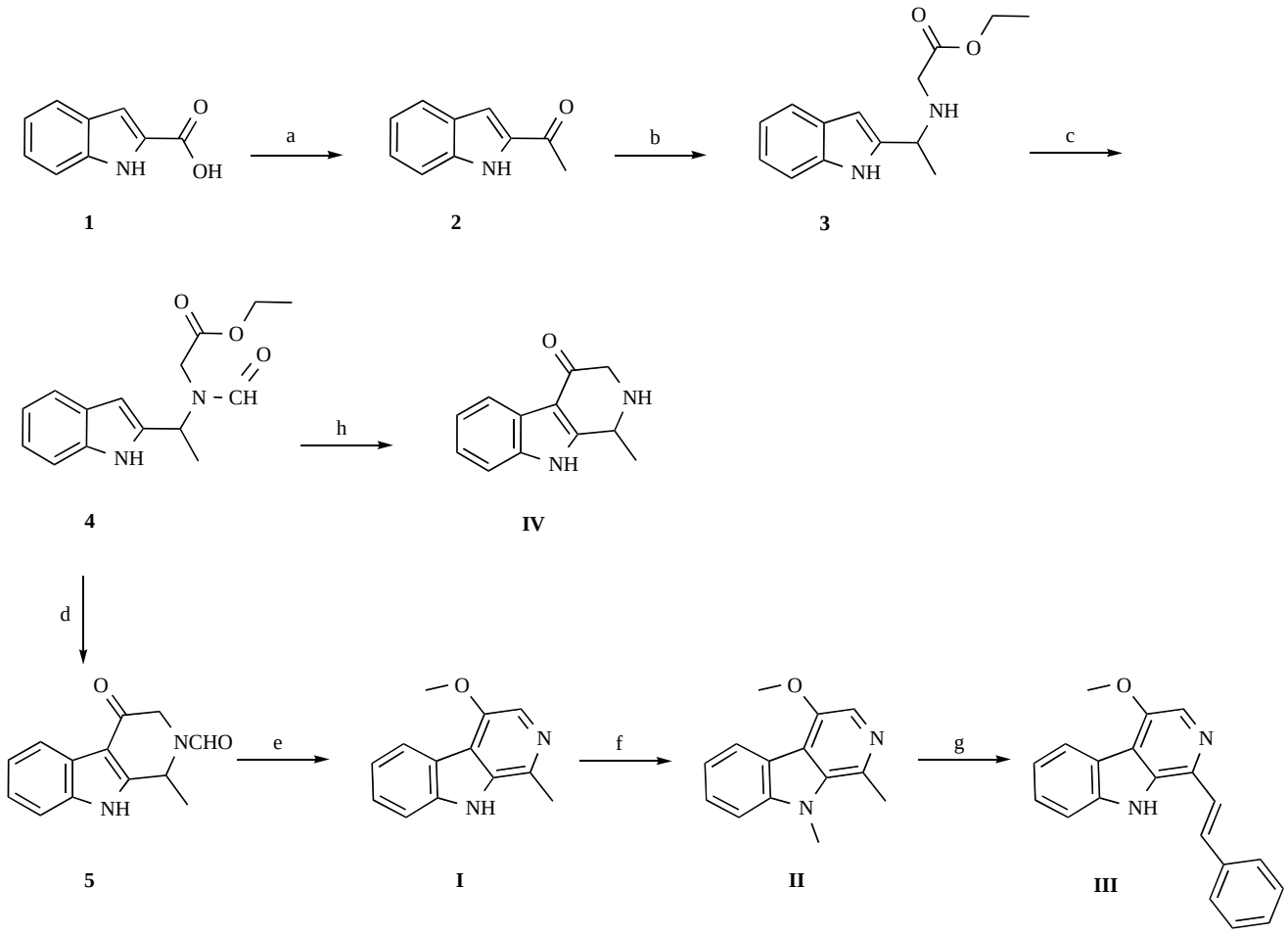
The preparation of derivatives **I-IV** is summarized in Scheme **1**. Treatment of indole-2-carboxylic acid (**1**) with a solution of methyllithium in ether afforded 2-acetylindole (**2)** in very good yield. **2** was converted to the glycine derivative (**3**) *via* reductive amination, using sodium triacetoxyborohydride as reducing agent in presence of triethylamine and acetic acid. Ethyl 2-[1-(1H-indol-2-yl)ethylamino]acetate **3** was treated with ethylformate and formic acid to furnish the key intermediate **4**. Cyclization of **4** with methanesulfonic acid gave intermediate **5** in excellent yield. 4-Methoxy-1-methyl-β-carboline (**I**) was obtained in a one-step conversion. This reaction was achieved by treatment of **5** with 2,2-dimethoxypropane, dehydrogenation of intermediate with chloranil, followed by ready methanolysis of N-formyl pyridinium intermediate. Methylation of the N-9 position of compound **I** has been achieved by the action of sodium hydride in a mixture of anhydrous DMF/THF followed by addition of methyl iodide, affording compound **II** in good yield. 1-Benzylidine substituted β-carboline **III** was readily prepared by reaction of 1,9-dimethyl-4-methoxy-β-carboline **II** with benzaldehyde in refluxing acetic anhydride.

Treating **4** with methanesulfonic acid 70% in water we obtained compound **IV** in which cyclization and deformylation were accomplished simultaneously.

Compounds **V, VI, VII, VIII**, and 1-methoxycanthin-6-one (**IX**) were prepared according to the literature procedures [28, 29]. 3-benzyl-1-methoxycanthin-6-onium bromide **(X)** was synthesized from 1-methoxycanthin-6-one by the simple quaternarization with benzyl bromide in refluxing ethyl acetate (Scheme **2**).

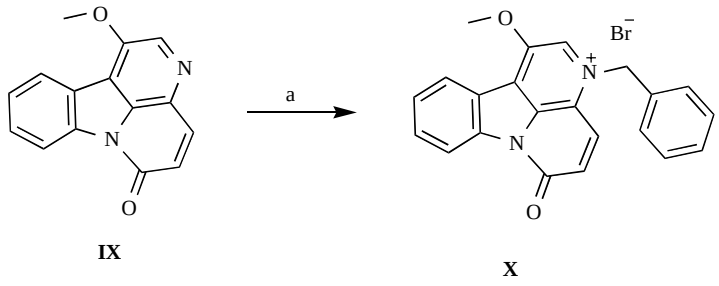
Antiproliferative Activity

The cytotoxic potential of all synthesized compounds was evaluated *in vitro* against a panel of human tumor cell lines. The tumor cell line panel consisted of human T cell lymphoblast-like cells (Jurkat), human breast cancer (MCF-7), human colon carcinoma (HT-29), human lung adenocarcinoma epithelial (A549), human prostate cancer (PC3), human melanoma (M14), human anaplastic thyroid carcinoma (ARO), and human glioblastoma (T98G). IC_{50}



Reagents and conditions: a) MeLi 1.6M in ether, THF, rt, o.n., 84%; b) glycine ethyl ester hydrochloride, $NaBH(OAc)_3$, TEA, AcOH, CH_2Cl_2, rt, 22 h, 89%; c) HCO_2Et, HCOOH, rt, 12 h, 94%; d) $MeSO_3H$, 70°C, 1h, 96%; e) $Me_2C(OMe)_2$, p-TsOH, benzene; then p-chloranil, rt, 12h, 50%; f) , CH_3I, NaH, THF-DMF, rt, 30', 80%; g) $(CH_3CO_2)O$, C_6H_5CHO, reflux, 24h, 67%; h) $MeSO_3H$ 70%in H_2O, 70°C, 1h, 95%.

Scheme 1. Synthesis of compounds **I-IV**.



Reagents and conditions: a) BnBr, AcOEt, reflux, 7h, 67%.

Scheme 2. Synthesis of compound **X**.

values for **I-X** and harmine (for comparison) are reported in Table **1**.

In this study we evaluated cytotoxic effects of β-carboline derivatives and compared with harmine, a natural alkaloid highly cytotoxic against human tumor cell lines. In addition, 1-methoxycanthin-6-one (**IX**) was tested for cytotoxicity against a panel of several tumor cells. Our experiments confirmed the cytotoxic effect of 1 methoxycanthin-6-one (**IX**) and, for the first time, we demonstrated the potential cytotoxic activity of compounds **I-VIII** and **X** in different cancer cell lines. From the above data, the following conclusions were drawn.

Table 1. ***In Vitro*** **Antitumor Activity of Derivatives I-X**

Compounds	IC_{50} (μM)[a]							
	M14	**MCF-7**	**HT-29**	**A549**	**PC-3**	**Jurkat**	**ARO**	**T98G**
harmine	n.s.[b]	n.s.[b]	79.4±0.9	n.s.[b]	22±0.8	n.s.[b]	26.5±0.7	35±1.1
I	n.s.[b]	n.s.[b]	50±1.5	80±0.5	20±1.2	n.s.[b]	50±0.8	n.s.[b]
II	36±0.6	32±0.1	27±0.2	50±0.4	22±1.2	65±0.8	30±0.5	40±1.2
III	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	60±0.9	n.s.[b]	n.s.[b]	n.s.[b]
IV	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	24±0.8	n.s.[b]	18±0.6	n.s.[b]
V	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	64±1.2	n.s.[b]
VI	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]
VII	n.s.[b]	n.s.[b]	n.s.[b]	n.s.[b]	50±0.6	n.s.[b]	n.s.[b]	n.s.[b]
VIII	38.9±0.1	35±0.45	90±0.3	45±1.2	25±0.2	28.8±0.9	39±0.4	n.s.[b]
IX	50±1.1	50±0.3	31±0.1	n.s.[b]	26±0.1	50±0.2	15±0.6	90±0.7
X	37±0.3	46±0.75	72±0.4	n.s.[b]	8±0.9	n.s.[b]	28±1.0	n.s.[b]

[a]IC_{50}= compound concentration required to inhibit tumor cell proliferation by 50%; [b]n.s. not significant. Data represent the mean values of three independent determinations performed in triplicate.

The shift of methoxy group from position 7 to 4 (harmine *vs* **I**) led to an increase of activity against HT-29 and A549 cell lines while no significant activities were detected in the other cell lines. The introduction of methyl group into position-9 of compound **I** led to compound **II** which demonstrated an improvement of activity to all tumor cell lines. When methyl group in position 1 was replaced with benzylidine substituent there is a loss of activity. Compounds **V** and **VI,** with no substituent at position-1, were inactive to all tumor cell lines. Interestingly, the compound bearing a carboxyaldehyde substituent in position-1 (**VIII)** of β-carboline ring system displayed a strong anti-proliferative effect against almost all tumor cell lines. In addition, the compound with a carboxylate (**VII**) substituent in position-1 was inactive to all tumor cell lines except to PC-3. Tetrahydro-β-carboline derivative **IV** showed good activity only against PC-3 and ARO cells.

As almost of the synthesized compounds of the present study showed interesting anticancer activity especially against PC-3 and ARO cell lines, oral bioavailability was considered to play an important role for the development of bioactive molecules as therapeutic agents. Therefore, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, topological polar surface area (TPSA), absorption (% ABS) and simple molecular descriptors used by Lipinski in formulating his ''rule of five''[30]. Calculations were performed using Molinspiration online property calculation toolkit [31]. Table **2** represents a calculated percentage of absorption (% ABS), topological polar surface area (TPSA) and Lipinski parameters of the synthesized compounds. Percentage of absorption (% ABS) was estimated using the equation: % ABS = 109 - 0.345 x TPSA, according to Zhao *et al.* [32]. TPSA was also calculated using Molinspiration online property calculation toolkit [31] according to the fragment-based method of Ertl *et al.* [33]. Polar surface area, together with lipophilicity, is an important property of a molecule in transport across biological membranes. Too high TPSA values give rise to a poor bioavailability and absorption of a drug. According to the above criterions, calculated percentages of absorption for compounds ranged between 87 and 99%.

As a whole, the present results showed that all the compounds studied, except **V** and **VI**, showed a very selective activity against prostate cancer cells PC-3 at IC_{50} values nearly to 20 μM. In particular, **X** demonstrated an important anti-proliferative effect at low concentration (IC_{50} 8 μM). Moreover the most active compounds **II, VIII, IX** and **X** appear to be suitable as leads for further anticancer molecules development efforts on the fact their size and chemical properties are appropriate to classify them as drug-like compounds as they follow all the Lipinski's rule of 5.

CONCLUSION

In conclusion, a number of novel β-carboline derivatives described in this paper proved to be selective and potent agent against prostate cancer. This important first step will allow us to determine preliminary relationship between structure and cytotoxic activities. The *in vitro* experiments revealed that the shift of methoxy group in position 4 of β-carboline ring of harmine led to enhanced cytotoxic activity, substituents at position-1 were essential for high activity towards specific tumor types. From the above data we have also demonstrated that substitution at the 3-position of 1-methoxycanthin-6-one with benzyl increased activity against most of the cell lines tested.

Ongoing studies will probe the mechanism of action of these new derivatives but the data presented in this paper already suggest that several derivatives are potential

Table 2. Predicted ADME, Lipinski Parameters and Molecular Properties of the Synthesized Compounds I-X and Harmine[a]

Cpd	% ABS	TPSA	n-ROTB	n-OHNH donors	n-ON acceptors	mi LogP	Mol. Wt	*n* violations
harmine	95	37.92	1	1	3	2.626	212.25	0
I	95	37.92	1	1	3	2.796	212.25	0
II	99	27.06	1	0	3	2.864	226.28	0
III	99	27.06	3	0	3	5.192	314.39	1
IV	93	44.89	0	2	3	1.829	200.24	0
V	96	37.92	1	1	3	2.575	198.23	0
VI	92	50.48	1	1	4	0.943	214.22	0
VII	87	64.22	3	1	5	2.646	256.26	0
VIII	90	54.99	2	1	4	2.604	226.24	0
IX	94	43.61	1	0	4	2.891	250.26	0
X	97	34.61	3	0	4	0.816	341.39	0

[a] % ABS: Percentage of absorption, TPSA: topological polar surface area, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, n-ROTB: number of rotatable bonds. Calculations were performed using *Molinspiration online property calculation toolkit* (http://www.molinspiration. com).

candidates for clinical development with models of prostate cancer.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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