β-Carboline Alkaloids: Biochemical and Pharmacological Functions

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Abstract: β-Carboline alkaloids are a large group of natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants, foodstuffs, marine creatures, insects, mammalians as well as human tissues and body fluids. These compounds are of great interest due to their diverse biological activities. Particularly, these compounds have been shown to intercalate into DNA, to inhibit CDK, Topisomerase, and monoamine oxidase, and to interact with benzodiazepine receptors and 5-hydroxy serotonin receptors. Furthermore, these chemicals also demonstrated a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, anticonvulsant, antitumor, antiviral, antiparasitic as well as antimicrobial activities. In this review, we summerized the biochemical and pharmacological functions of β-carboline alkaloids.

Keywords: Reviews, β-carbolines, biochemical, pharmacological, structrue-activity relationship.

1. INTRODUCTION

The β-carboline alkaloids are a large group of natural and synthetic indole alkaloids that possess a common tricyclic pyrido[3,4-b]indole ring structure [1, 2]. These molecules can be categorized according to the saturation of their N-containing, six-membered ring. Unsaturated members are named as fully aromatic β-carbolines (βCs), whereas the partially or completely saturated ones are known as dihydro-β-carbolines (DHβCs) and tetrahydro-β-carbolines (THβCs), respectively. Those tricyclic compounds usually contain several substituents both in the pyrido ring and/or the indole ring. The so-called Pictet-Spengler condensation reaction of indoleethylamines or tryptophan with aldehyde or α-keto acids has been proven to be the most efficient route for the chemical synthesis of THβCs or tetrahydro-β-carboline-3-carboxylic acids (THβCAs) (Fig. **1**) and the reaction readily occurs under mild conditions and is temperature and pH-dependent. And their photophysical properties are strongly affected by the presence of two different nitrogen atoms in the tricyclic system, the pyridinic and the pyrrolic nitrogens. The pyridinic nitrogen is more basic than the pyrrolic one, while its basicity increases upon excitation [3, 4] and is affected by the substituents presence in the structure [5]. Depending upon pH and solvent, β-carbolines can exist in four forms [6]: the cation, the neutral form, a zwitterion (or an alternative quinine-type canonical form), and an anion.

The β-carboline alkaloids 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 [209]. 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 [211]. During the last two decades, numerous simple and complicated β-carboline alkaloids with saturated or unsaturated tricyclic ring system have been isolated and identified from various terrestrial plants as the major bioactive constituents. Reports up to 2003 on the isolation and characterization of simple β-carboline alkaloid including norharman and harman were summarized [2, 210]. Numerous reports also disclosed that other simple and complicated β-carboline alkaloids were extensively presented in extracts from the leaves, barks and roots of a variety of plants.

In addition, numerous simple or intricate β-carboline alkaloids have been isolated and characterized from various marine invertebrates, which include hydroids [212] (*Aglaophenia*), bryozoans [174,213,214] (*Cribricellina*, *Catenicella*), soft corals [215] (*Lignopsis*), tunicates [216-226] (*Eudistoma*, *Didemnum*, *Lissoclinum*, *Ritterella*, *Pseudodistoma*), and various sponges. Marine ascidians belonging to the genus Eudistoma (family Polycitoridae) are another rich source of biologically active β-carboline derivatives. So far, numerous β-carboline alkaloids, including eudistomins A-T [177,178,227,228], eudistomidins A-F [229-231], eudistalbins A and B [236], eudistomin U and isoeudistomin U [222], eudistomin V [225] and two new trypargine derivatives [233], have been isolated and characterized from various Eudistoma species.

It is well-known that the simple β-carboline alkaloids, such as tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-tetrahydro-β-carboline-3-carboxylic acid, are easily formed from tryphtophan or tryptamine and formaldehyde or pyruvate or acetate precursors by known Pictet-Splengler reaction in foods and berverages. During the last two decades, it had been demonstrated that various tetrahydro-β-carboline and β-carboline alkaloids in variable but apprecia-

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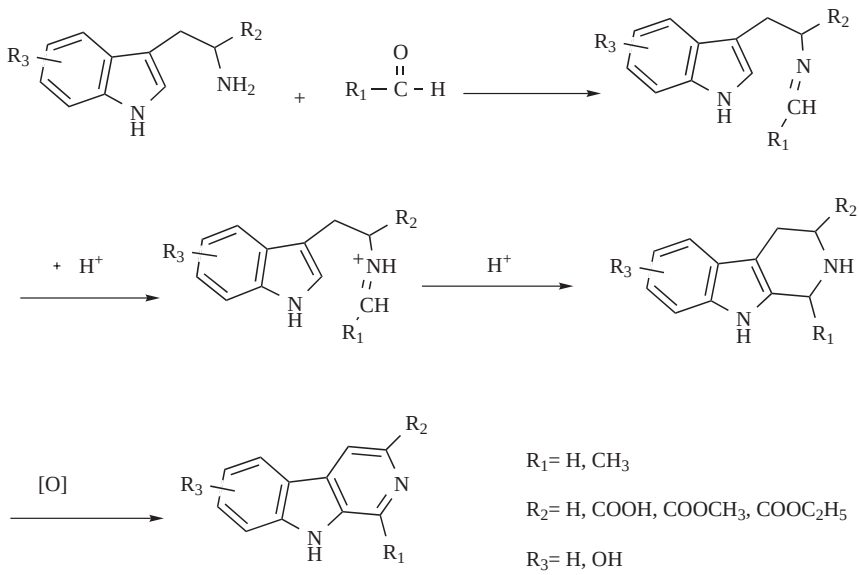


Fig. (1). Pictet-Spengler condensation between indoleamines and acetaldehyde or formaldehyde to give simple tetrahydro-β-carboline (THβCs) alkaloids. Oxidation of THβCs provides β-carbolines.

ble levels were widely found in foods, alcoholic and nonalcoholic beverages, and fruit and fruit-derived products.

The occurrence of β-carboline and related β-carboline alkaloids in commonly ingested foodstuffs strongly proved that diet is an important exogenous source of these compounds in mammals and humans. Undoubtedly, the ingestion of these compounds during foods and beverages comsumption that could be partially responsible for their further endogenous presence in various mammals' tissues, organs and physiological fluids [235-239] besides certain endogenous formation itself by putative biosynthesis pathway.

Since the first time in 1961, McIsaac [234] isolated and identified endogenous pinoline (6-methoxy-tetrahydro-β-carboline, **10**) from an extract of pineal gland tissue, extensive investigations have focused on the detection and identification of β-carboline alkaloids in mammals. At the present time, researches clearly confirmed that numerous β-carboline alkaloids − norharman (**1**), harman (**2**), harmine (**3**), β-CCE (**11**), harmaline (**6**), harmalan (**9**), and several different tetrahydro-β-carbolines − distributed widely in various tissues and fluids of a variety of mammals.

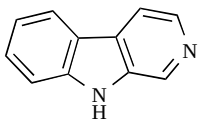
Previously, numerous reports investigated the effects of β-carboline alkaloids on the central nervous system (CNS), such as their affinity with benzodiazepine receptors (BZRs), 5-HT_{2A} and 5-HT_{2C} receptors [7-9]. However, recent interest in these alkaloids has been focused on their potent antitumor, antiviral, antimicrobial and antiparasitic activities. Here, we present a brief, yet comprehensive, up-to-date summary with a special emphasis on the biochemical and pharmacological importance of β-carboline alkaloids.

2. BIOCHEMICAL EFFECTS OF β-CARBOLINES

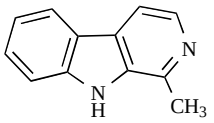
2.1. Interaction with DNA

Numerous investigations have shown that the biological and pharmacological effects of β-carbolines in prokaryotic and eukaryotic cells attributed in part to their ability of DNA intercalation leading to altered DNA replication fidelity or to an influence on enzymatic activities in DNA repair process [10-15]. Initially, Hayashi *et al*. [16] found that harman (**2**) and norharman (**1**) reacted with DNA by intercalation, resulting to a quenching of the fluorescence of norharman and a marked red shift and hypochromism of the absorption spectra of norharman (**1**) and harman (**2**). However, harman (**2**) intercalated more easily into DNA than norharman (**1**) [16]. Harmine (**3**) was also reported to interaction with the calf thymus DNA (CT-DNA) by intercalation. However, hydrogenation of a double bond of the pyridine ring that converts harmine (**3**) into harmaline (**6**) greatly altered the intercalation capacity of the molecule with DNA [17]. Harman (**2**) decreased the cellular capacity to repair DNA damage and to fix mutation in Chinese hamster cells [18] and induced sister-chromatid exchanges (SCE) in human peripheral lymphocytes *in vitro* [19].

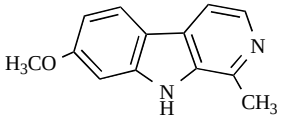
In addition, both harman (**2**) and norharman (**1**) inhibited the transcription of isolated DNA *in vitro* [19] and induced SOS responses as well as reversion of trpE9777 frameshift mutation in bacteria [20]. Mita *et al*. [21] reported that norharman (**1**) reduced the DNA strand breaks and mutation of Chinese hamster V79 cells by chemical carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), but enhanced the 1-oxide (4HAQO)-induced DNA strand breaks. Administration of 0.1% harman (**2**) to mice in their diet for 4 weeks resulted in DNA adducts in the liver and kidney, whereas similar treatment of mice with norharman (**1**) resulted in DNA adducts in the kidney, glandular stomach and large intestine, but not in the liver [22]. Harman (**2**), norharman (**1**) and its related β-carbolines harmine (**3**) and harmaline (**6**) inhibited DNA excision repair directly or indirectly, and consequently enhancing UV or chemically induced mutagenesis [23]. Moreover, harman (**2**) and harmine (**3**) induced chromosome aberrations in Chinese hamster ovary cells (CHO) after treatment with UV light and mitomycin C, in the presence of a metabolic activation system (S_9 mix) [24], and increased aberrant cell frequency and induced DNA damage were observed in V79 Chinese hamster lung fibroblasts *in vitro* [25].



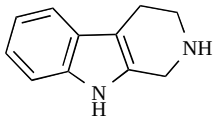
Norharman (1)



Harman (2)



Harmine (3)



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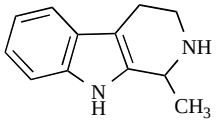
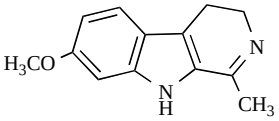
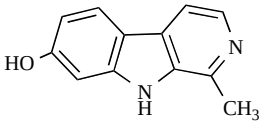


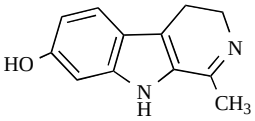
MTHβC (5)



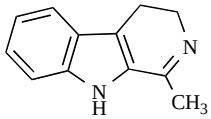
Harmaline (6)



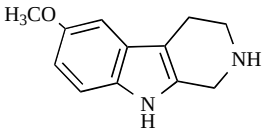
Harmol (7)



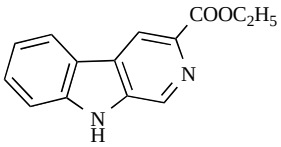
Harmalol (8)



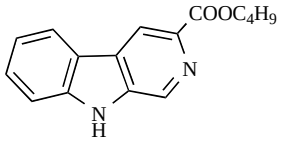
Harmalan (9)



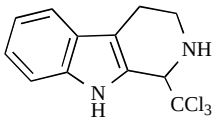
Pinoline (10)



βCCE (11)



βCCB (12)



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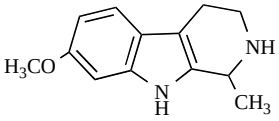
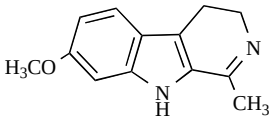
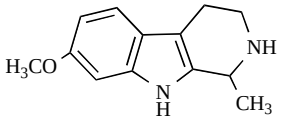


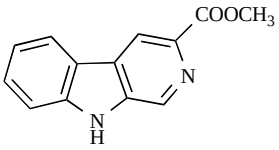
Tetrahydroharmine (14)



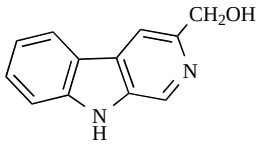
6-methoxyharmalan (15)



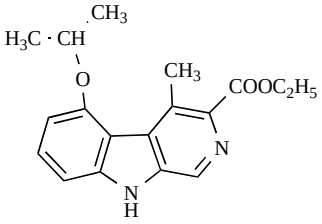
6-methoxytetrahydroharmalan (16)



βCCM (17)



3-HMC (18)



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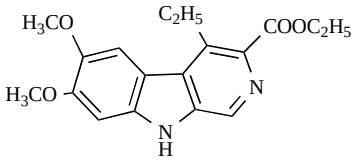

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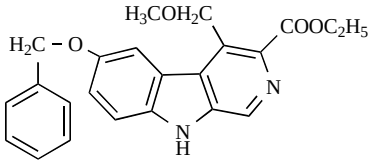

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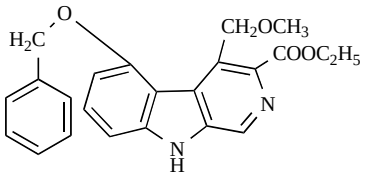

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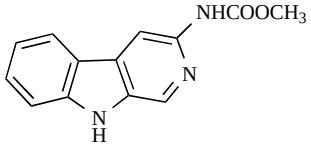

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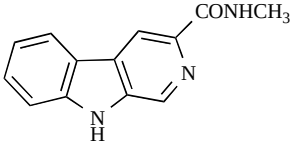

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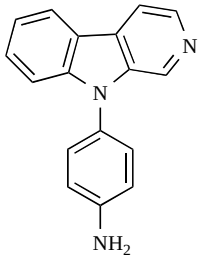

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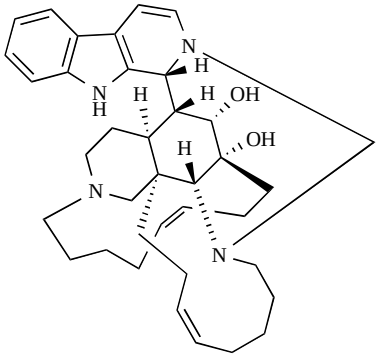
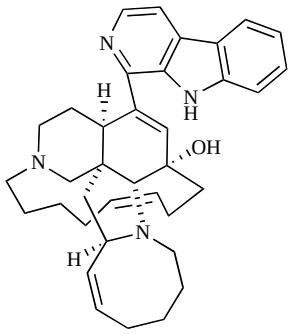
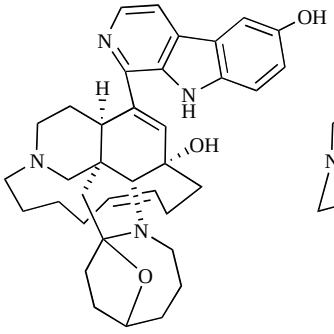


Ma'eganedin A (26)

(Fig. 2) contd…..



Manzamine A (27)



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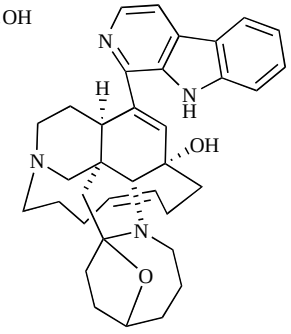
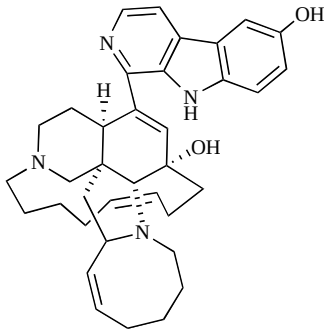


6-Deoxymanzamine X (29)



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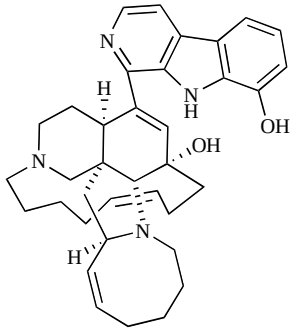

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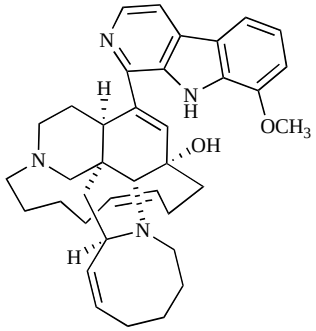

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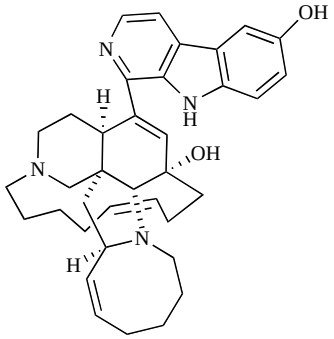

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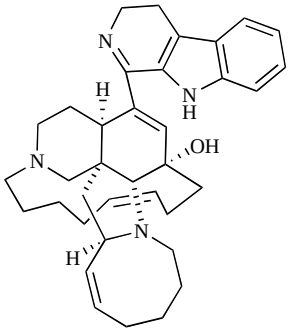
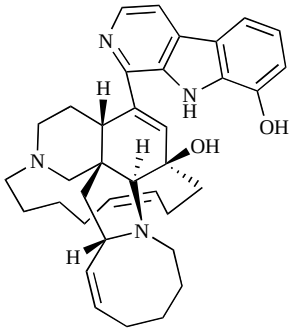
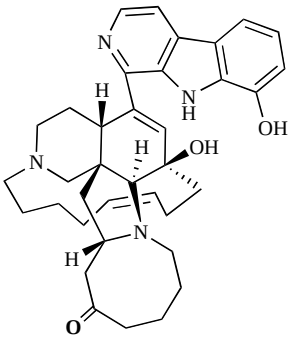


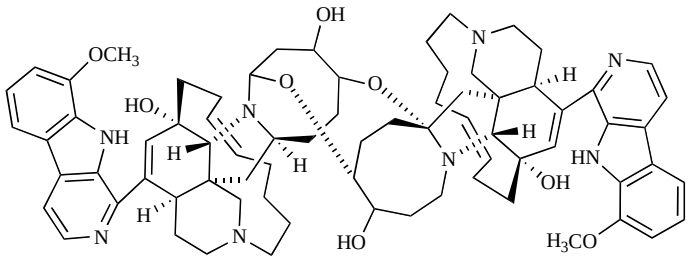
3,4-Dihydromanzamine A (34)



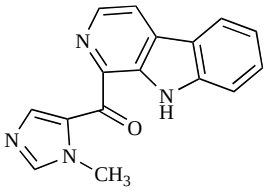
Ent-8-hydroxymanzamine A (35)



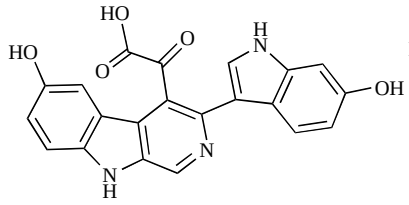
Ent-manzamine F (36)



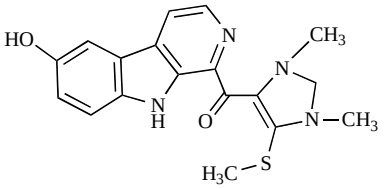
Neo-kauluamine (37)



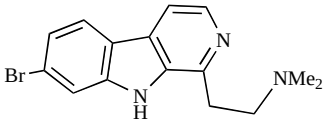
Xestomanzamine B (38)



Hyrtioerectines A (39)

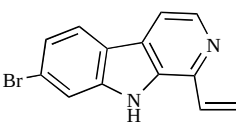


Gesashidine A (40)

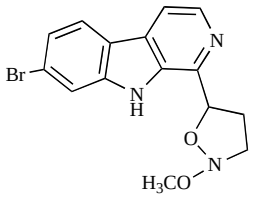


Plakortamines A (41)

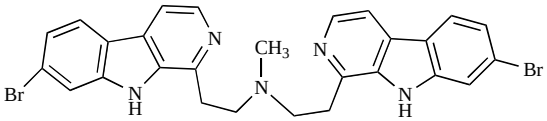
(Fig. 2) contd…..



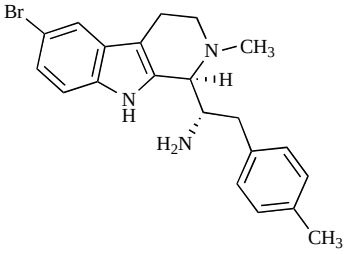
Plakortamines B (42)



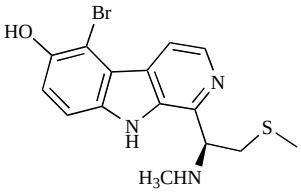
Plakortamines D (43)



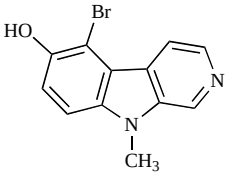
Plakortamines C (44)



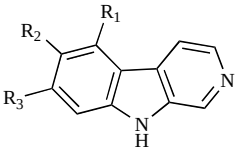
Eudistomidins B (45)



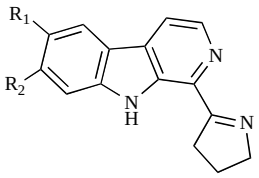
Eudistomidins C (46)



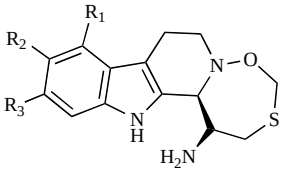
Eudistomidins D (47)



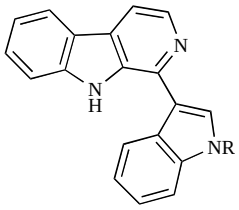
Eudistomin D R_1=Br R_2=OH R_3=H (48)
Eudistomin N R_1=H R_2=Br R_3=H (49)
Eudistomin O R_1=H R_2=H R_3=Br (50)



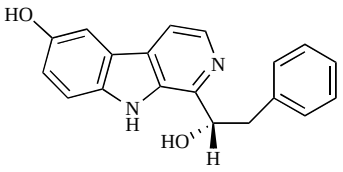
Eudistomin H R_1=Br R_2=H (51)
Eudistomin I R_1=H R_2=H (52)
Eudistomin P R_1=OH R_2=Br (53)
Eudistomin Q R_1=OH R_2=H (54)



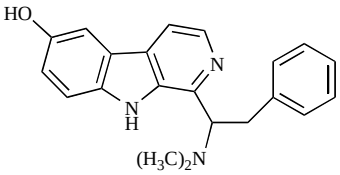
Eudistomin C R_1=H R_2=OH R_3=Br (55)
Eudistomin E R_1=Br R_2=OH R_3=H (56)
Eudistomin K R_1=H R_2=H R_3=Br (57)
Eudistomin L R_1=H R_2=Br R_3=H (58)
(-)-Debromoeudistomin K R_1=H R_2=H R_3=H (59)



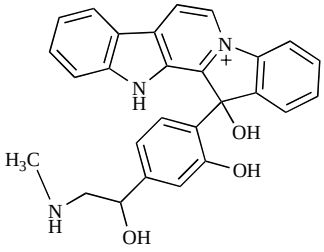
Eudistomin U (60)



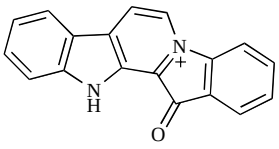
Eudistomin W (61)



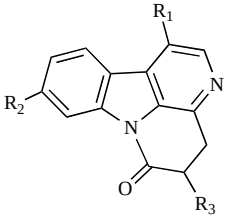
Eudistomin X (62)



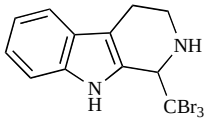
Threctandramine (63)



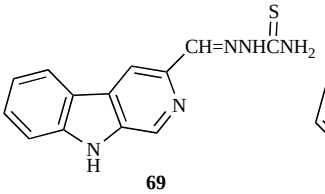
Fascaplysin (64)



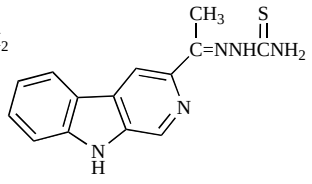
Canthin-6-one R_1=H R_2=H R_3=H (65)
1-Methoxycanthin-one R_1=OCH_3 R_2=H R_3=H (66)
5-Methoxycanthin-one R_1=H R_2=H R_3=OCH_3 (67)



TaBro (68)



69



70

Fig. (2). Chemical structure of the mentioned β-carboline alkaloids.

Recently, harman (**2**) and norharman (**1**) were demonstrated to induce DNA damage in a dose-dependent manner in SH-SY5Y cells [26]. The *Peganum harmala L*. seeds extract, in which harmine (**3**), harman (**2**) and harmaline (**6**) are the dominating components, were also found to interact with DNA [27]. Our group [28] reported that harmine (**3**) and its 9-substituted derivatives exhibited remarkable DNA intercalation activities and the potency of intercalation into DNA were enhanced significantly by introducing an appropriate substituent into position-9 of β-carboline nucleus. Moreover, 3-substituted β-carboline derivatives interacted with CT-DNA *via* intercalation mode [29], and β-carboline derivatives bearing guanidinium group or amino group-terminated side chain blocked the Tat-TAR interaction, which underlies its anti-HIV activities [30].

Furthermore, the potent dopaminergic neurotoxin 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo, **21**) was found to induce cell-free plasmid DNA damage *via* single-strand scissions mode [31], and the damage effects of TaClo (**21**) towards cell-free DNA was also observed in the presence of Cu (II) [223]. Toshima *et al*. [32] reported that β-carboline-carbohydrate hybrid molecules as a novel DNA photocleaver selectively cleaved the cell-free DNA at the guanine site upon irradiation with UV light with a long wavelength without any additive. Harmine (**3**) and its 9-substituted derivatives were also potential photocleavers to cell-free plasmid DNA, and the potency of cleavage of those chemicals towards DNA was increased dramatically by introducing electron-releasing substituent into β-carboline nucleus [28, 193].

Table 1. The Interaction of the β-Carbolines with DNA

Compounds investigated	Interaction with DNA
Norharman	1. Intercalation into DNA [16]; 2. Inhibition the transcription of isolated DNA [19]; 3. Induction SOS response [19]; 4. Induction reversion of trpE9777 frameshift mutation [19]; 5. Reduction the DNA strand break and mutation induced by MNNG while enhanced the DNA strand break induced by 1-oxide(4HAQQ) [21]; 6. Formation DNA adducts [22]; 7. Inhibition DNA excision repaired [23]; 8. Induction DNA damage [26];
Harman	1. Intercalation into DNA [16, 27]; 2. Decrease the capacity of repair DNA damage [18]; 3. Induction SCE [19]; 4. Inhibition the transcription of isolated DNA [19]; 5. Induction SOS response [19]; 6. Induction reversion of trpE9777 frameshift mutation [19]; 7. Formation DNA adducts [22]; 8. Inhibition DNA excision repaired [23]; 9. Induction chromosome aberration in CHO cells [24]; 10. Increase aberrant cell frequency [25]; 11. Induction DNA damage [25,26];
Harmine	1. Intercalation into DNA [16, 27, 28]; 2. Inhibition DNA excision repaired [23]; 3. Induction chromosome aberration in CHO cells [24]; 4. Increase aberrant cell frequency [25]; 5. Scission DNA upon irradation with UV light [28];
Harmaline	1. Intercalation into DNA [27]; 2. Inhibition DNA excision repaired [23];
9-Substituted harmine derivatives	1. Intercalation into DNA [28]; 2. Scission DNA upon irradation with UV light [28];
3-Substitued β-carboline derivatives	1. Intercalation into DNA [29]; 2. Block the Tat-TAR interaction [30];
TaClo	Induction cell-free plasmid DNA damage [31];
β-Carboline-carbohydrates	Scission DNA upon irradation with UV light [32];
APNH	1. Formation DNA adducts [34]; 2. Induction SCE and chromosome aberrations [35];

Aminophenylnorharman (APNH, **25**), a newly identified mutagenic heterocyclic amine formed by coupling of norharman (**1**) with aniline in the presence of S_9 mix, was N-hydroxylated to N-hydroxy-aminophenylnorharman (N-OH-APNH) in metabolic system [33] followed by the formation of DNA adducts, and consequently caused oxidative DNA damage [34]. APNH (**25**) induced SCE and chromosome aberrations in cultured Chinese hamster lung cells (CHL) [35] and the action at the DNA levels by APNH (**25**) is probably due to reaction with guanine to ultimate forms derived from N-OH-APNH, which is converted from APNH (**25**) through N-hydroxylation by P-450 enzymes [36]. When APNH (**25**) administered to F344 rats at a dose of 40 ppm for 4 weeks, the DNA adduct, confirmed to be dG-C8-APNH, was detected in all organs including the liver, colon and lung [37].

Interestingly, β-carboline-3-carboxaldehyde thiosemicarbazone (**69**) was found to preferentially block DNA synthesis by inhibiting ribonucleoside diphosphate reductase and, consequently, inhibiting the incorporation of thymidine into DNA, while its effect on RNA and protein synthesis was much less pronounced. Of particular interest, its analogue 3-acetyl-β-carboline thiosemicarbazone (**70**) was observed to have little or no effect on DNA synthesis, and it might be an

Table 2. The Interaction of the β-Carbolines with Enzymatic Systems

Compounds investigated	Interaction with enzymatic systems
Norharman	1. Inhibition of Topo I [11, 27] and Topo II activities [11]; 2. Inhibition of 5-HT uptake [42]; 3. Inhibition of MAO-B activity [43, 44, 48]; 4. Interaction with CYP11 and CYP17 [51]; 5. Inhibitors of heme containing protein indoleamine 2,3-dioxygenase [52]; 6. Interaction with the CYP enzymes [50]; 7. Inhibition of the activity of CYP-related enzyme [53]; 8. Inhibition of 2-acetylaminofluorene N-hydroxylase [47]; 9. Inhibition of aldehyde oxidase [55]; 10. Inhibition of benzo[a]pyrene metabolism by MFO [56,57]; 11. Inhibition of mono-oxygenase [58]; 12. Stimulation of epoxide hydrolase activity [58]; 13. Bound with high affinity to GRP 78 and carboxylesterase [60]; 14. Bound with high affinity to triosephosphate isomerase [61];
Harman	1. Inhibition of the AP endonuclease activity of phage T4 [38]; 2. Inhibition of Topo I [11, 27] and Topo II activities [11]; 3. Inhibition of MAO-A activity [43, 44, 48];
Harmine	1. Inhibition of human DNA Topo I activity [27, 28]; 2. Inhibition of 5-HT uptake [42]; 3. Inhibition of MAO-A activity [49]; 4. Potent and specific inhibitors of CDKs [62, 63];
Harmaline	1. Inhibition of human DNA Topo I activity [27]; 2. Interaction with cation in Na^+-independent dibasic amino acid transport system [40]; 3. Inhibition of Na^+-dependent I-uptake [41]; 4. Inhibition of PKC activity [204] 5.
TaClo	1. Inhibition of TH activity [66]; 2. Inhibition complex I of the mitochondrial respiratory chain [196]
TaBro	Inhibition complex I of the mitochondrial respiratory chain [196]
βCCB	Activation of the mitochondrial pathway [67];
βC+	1. Substrate for the dopamine transporter [68, 69]; 2. Interaction with specific cellular targets such as protein [110]; 3. Interaction with Parkinson's disease-related proteins [72];
Other β-carboline derivatives	1. Inhibition of CDKs activities [62, 63]; 2. Bound with TPI [61]; 3. Inhibition of PDEs activities [64]; 4. Inhibition of IKK activities [65]; 5. Inhibition of Topo II-mediated DNA relaxation/ cleavable complex formation [39]; 6. Inhibition of tubulin polymerization [39]. 7. Inhibition of PDGFs receptor kinase [208]

inhibitor of RNA and protein synthesis, and the mechanism of action was poorly understood.

2.2. Interaction with Enzymatic Systems

Different β-carboline alkaloids seem to interact selectively with specific enzymatic systems leading to a variety of pharmacological activities. Warner *et al.* [38] reported that harman (**2**) selectively inhibited the apurinic/apyrimidinic (AP) endonuclease activity of phage T4-induced UV endonuclease. The DNA relaxation activity of Topo I and Topo II was effectively inhibited by harman (**2**) and norharman (**1**) at 1-10μg/ml and 20-250μg/ml, respectively [11]. However, harman (**2**) and norharman (**1**) did not inhibit DNA cleavage activities of Topo I and Topo II nor stabilize the cleavable complex by Topo I and Topo II [11]. The *Peganum harmala L*. seeds extract, in which harmine (**3**), harman (**2**) and harmaline (**6**) are the dominating components, were also found to inhibit human DNA Topo I activity [27]. Noticeably, our group [28] reported that harmine (**3**) and its 9-substituted derivatives exhibited remarkable Topo I inhibition activity but no effect with Topo II, and the potency of Topo I inhibition were enhanced significantly by introducing an appropriate substituent into position-9 of β-carboline nucleus, the electron-releasing groups were favorable, while the electron-withdrawing substituent was detrimental. Besides, amino acid functionalized β-carboline derivatives significantly inhibited Topo II-mediated DNA relaxation and Topo II-mediated cleavable complex formation or tubulin polymerization [39].

Harmaline (**6**) interactions with cation in Na^+-independent dibasic amino acid transport system in human erythrocytes were observed [40]. Na^+-dependent I-uptake was inhibited by harmaline (**6**) [41]. Norharman (**1**), harmine (**3**) and other related β-carbolines were potent inhibitors of 5-hydroxytryptamine-induced human platelet aggregation, which inhibited 5-hydroxytryptamine uptake of human platelet at a lower concentration of monoamine A [42]. Norharman (**1**) and harman (**2**) were reversible competitive monoamineoxidase (MAO) inhibitors [43-46], and norharman (**1**) inhibited preferentially MAO-B, whereas harman (**2**) inhibited MAO-A [44]. What's more, substantial evidence has confirmed that β-carboline alkoliods occurring in tobacco smoke [48] and coffee [43] such as norharman (**1**) and harman (**2**) were responsible for the smoking and coffee drinking-linked reduction of MAO-A and B in smokers and in regular coffee drinkers. Harmine (**3**) and related β-carboline derivatives [49] were also reversible competitive inhibitors selective for MAO-A, and harmine (**3**), 2-methylharminium, 2,9-dimethylharminium and harmaline (**6**) were the most effective inhibitors of the purified MAO-A, and its potency of inhibition were increased by introducing 1-methyl and 7-methoxy substituents into tricyclic ring system.

Cytochrome P450 (CYP) isozymes are expressed and active not only in liver, but also in extrahepatic tissues, including brain. These CYP isozymes are known as the key enzymes in the metabolic activation of chemical carcinogens and toxins [50]. Kuhn Velten [51] reported that norharman (**1**) interacted with the steroidogenic CYP 11 in rat adrenal mitochondria and CYP 17 in rat testicular microsomes and progesterone binding to CYP17 was competitively inhibited by norharman (**1**), while harman (**2**), tetrahydro-β-carboline (THβC, **4**) and 1-methyl-1,2,3,4-tetrahydro-β-carboline (MT HβC, **5**) had no effect up to a concentration of 200 uM. Norharman (**1**) is one of the few known inhibitors of heme comtaining protein indoleamine 2,3-dioxygenase [52], so it is reasonable to speculated that norharman (**1**) acts as an inhibitor of the heme containing CYP-related enzymes [53]. Stawowy *et al.* [50] found that norharman (**1**) bound with high affinity to the CYP enzymes, such as CYP2E1, CYP1A1/2 and CYP2A6, leading to the activation of many promutagens to mutagens and carcinogens. Other studies, however, have shown that norharman (**1**) inhibited the activity of CYP-related enzymes, such as ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MR OD), pentoxyresorufin O-dependentylase (PROD), aniline hydroxylase (AH), 4-nitrophenol hydroxylase (NPH) and aminopyrine demethylase (APDM), through direct interaction with the O_2-binding site of the CYP heme iron [53]. Recently, investigations clearly demonstrated the involvement of CYP enzymes, such as CYP1A1, CYP1A2, CYP 2C9, CYP2C19 and CYP2D6, in the metabolic O-demethylation of harmaline (**6**) and harmine (**3**). Among them, CYP1A2 and CYP2D6 were the major P450 isozymes contributing their metabolic functions. Thus, O-demethyl-ation of these β-carbolines may be an important detoxication process protecting neurons from chemical damage [54]. Interestingly, the inhibition effect of harmaline (**6**) on Leish-mania protein kinase C (PKC) activities was also observed [204].

In addition, norharman (**1**) inhibited 2-acetylaminofluorene (AFF) N-hydroxylase [47], aldehyde oxidase [55], benzo[a]pyrene (BP) metabolism by MFO [56-57], monooxygenase [58]. In contrast, norharman (**1**) stimulated epoxide hydrolase activity [58]. Norharman (**1**) caused a decrease in the activity of mouse and rat microsomal monooxygenases, but has no effect on the activity of NADPH P450 reductase of epoxide hydrolase [59]. Interestingly, norharman (**1**) was found to bind with high affinity to certain proteins isolated from rat liver, such as the chaperone member glucose regulated protein 78 (GRP 78) and the enzyme carboxylesterase [60]. Bonnet *et al.* [61] reported that norharman (**1**) and related β-carboline derivatives bound with high affinity to triosephosphate isomerase (TPI) isolated from bovine brain and 2,9-dimethylnorharman was the most potent inhibitors of TPI. Thus β-carboline derivatives caused chronic neurodegeneration by inhibiting TPI and subsequently modulated the glycolytic pathway.

Recently, harmine (**3**) and numerous related β-carboline derivatives were found as potent and specific inhibitors of cyclin-dependent kinases (CDKs) [62-63]. It is worthy noted that harmine (**3**) specifically inhibited CDK1, CDK2 and CDK5, and the structure activity relationships (SARs) analysis demonstrated that the degree of aromaticity of the tricyclic ring and the positioning of substituents were crucial for inhibitory activity [62-63]. In addition, N^2-furoyl and N^2-pyrimidinyl β-carbolines were found to strongly inhibit activity against phosphodiesterases (PDEs) [64]. Similarly, 5-bromo-6-methoxy-β-carboline and other related β-carboline derivatives were recently identified as novel IκB kinase (IKK) inhibitors [65]. Meanwhile, the potent dopaminergic neurotoxin TaClo (**13**) dose-dependently inhibited basal L-tyrosine tetrahydropteridine/oxygen oxidoreductase (TH) activity after enzyme activation by pituitary adenylate cyclase-activating polypeptide [66], while N-butyl-β-carboline-

3-carboxylate (βCCB, **12**) induced the apoptosis cell death of cultured cerebellar granule neurons (CGNs) by activating mitochondrial pathway [67]. What's more, TaClo (**13**) and its analogue 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaBro, **68**) were found to induce a severe impairment of the nigrostriatal dopamine metabolism and inhibit complex I of the mitochondrial respiratory chain highly selectively [196]. It is worthy noted that pyrrolo[3,4-c]-β-carboline-diones were observed to act as a novel tyrosine kinase inhibitors with the capacity to selectively interfere with platelet-derived growth factor (PDGF) receptor signal transduction and PDGF-dependent cell growth [208].

N-methylated β-carbolinium ions (βC+) are substrates for the dopamine transporter [68-69], and are potent inhibitors of mitochondrial respiration [70-71]. βC+ is postulated to interact with specific cellular targets such as various proteins [110]. Recently, Gearhart *et al.* [72] identified six different brain proteins, including dorfin, α-tubulin, paraoxonase 2, fatty acid binding protein 5, platelet-activating factor acetylhydrolase 1B1 and nucleolar phosphoprotein p130, by using the Phage Display System, and five of six 2-methylnorharman-interacting proteins might have relation to Parkinson's disease. Whether 2-methylnorharman affects the function of these specific proteins *in vitro* and *in vivo* remains to be further investigated.

2.3. Interaction with Receptors

In addition to interaction with enzymes systems, various receptor systems are also important protein targets for β-carboline derivatives to exhibit a variety of significant pharmacological effects. During the last two decades, numerous investigations have discovered that the β-carboline alkaloids had high affinity for 5-hydroxytryptamine (5-HT), dopamine (DA), benzodiazepine (BZ) and imidazoline receptors.

Various β-carboline alkaloids bind at BZRs and act as full, partial or mixed agonists, antagonists or inverse agonists, achieving either sedative, tremorgenic, anxiolytic, anxiogenic, proconvulsant or convulsant effect [73-78]. The structure-affinity analysis suggested that the presence of a 3-position substituent (e. g. amide, ester, carbinol) and a fully aromatic ring system are optimal for BZR binding; while the tetrahydro-β-carbolines demonstrated considerably less affinity for BZR than their fully aromatic counterparts [79-81]. In accordance with this rule, Glennon *et al*. [82] found that the DHβCs lacked the affinity for BZR, even when a 3-position ester group was incorporated into the ring of harmalan (**9**), the compound did not bind to BZRs [82].

Since Glennon [83] reported that β-carboline alkaloids bound at 5-HT receptors of isolated rat fundus tissue, many investigations have concentrated on the interaction between β-carbolines and 5-HT receptors. Based on the tetrahydro-β-carboline alkaloid yohimbine, Audia *et al*. [84] developed a series of potent, selective 5-HT_{2B} contractile receptor antagonists, and these high-affinity THβC (**4**) antagonists were able to discriminate among the 5-HT_2 families of serotonin receptors, with members of the series showing selectivities of more than 100-fold versus both 5-HT_{2A} and 5-HT_{2C} receptors. Glennon *et al*. [85] found that β-carbolines bound with modest affinity to 5-HT_{2A} receptors, which was highly dependent upon the degree of ring saturation and the presence and position of substituents. Similar conclusions were also drawn regarding 5-HT_{2C} binding to [^{3}H]ketanserin-labelled 5-HT_{2C} receptors [86]. In contrast, the βCs displayed low or no affinity for 5-HT_{1A} receptors [85]. Boksa and colleagues [87-91] synthesized a number of THβC (**4**) derivatives and evaluated their affinity with the 5-HT receptor system. They found that several compounds of these derivatives had mixed activities to 5-HT_{1A}/5-HT_{2A} receptors. Recently, a series of ring substituted DHβCs and THβCs were examined with 5-HT_{2A} and 5-HT_{2C} serotonin receptors, and the SARs analysis indicated that most of the methoxy-substituted derivatives typically displayed affinities similar to their unsubstituted parents, while certain bromo derivatives displayed enhanced affinity [83]. Certain THβC derivatives were also found to bind at 5-HT_{5A} receptors with modest affinity. Abdel-Fattah *et al*. [92] reported that total alkoloid extracted from *Peganum harmala* seeds and its major component, harmine (**3**) and harmaline (**6**), produced a dose-dependent hypothermia in rats mainly *via* endogenous 5-HT stimulation of the 5-

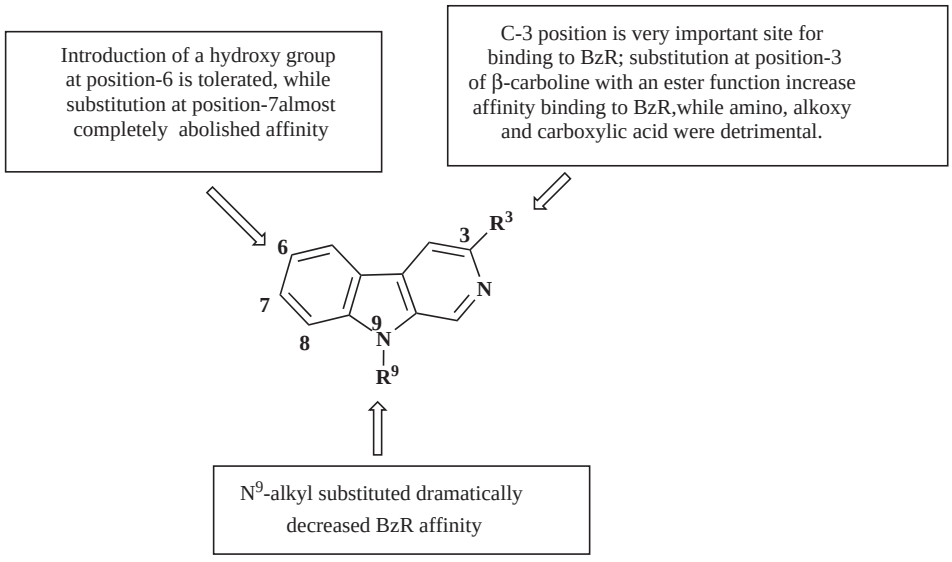


Fig. (3). The structure –affinity relationships of β-carbolines binding to BzR.

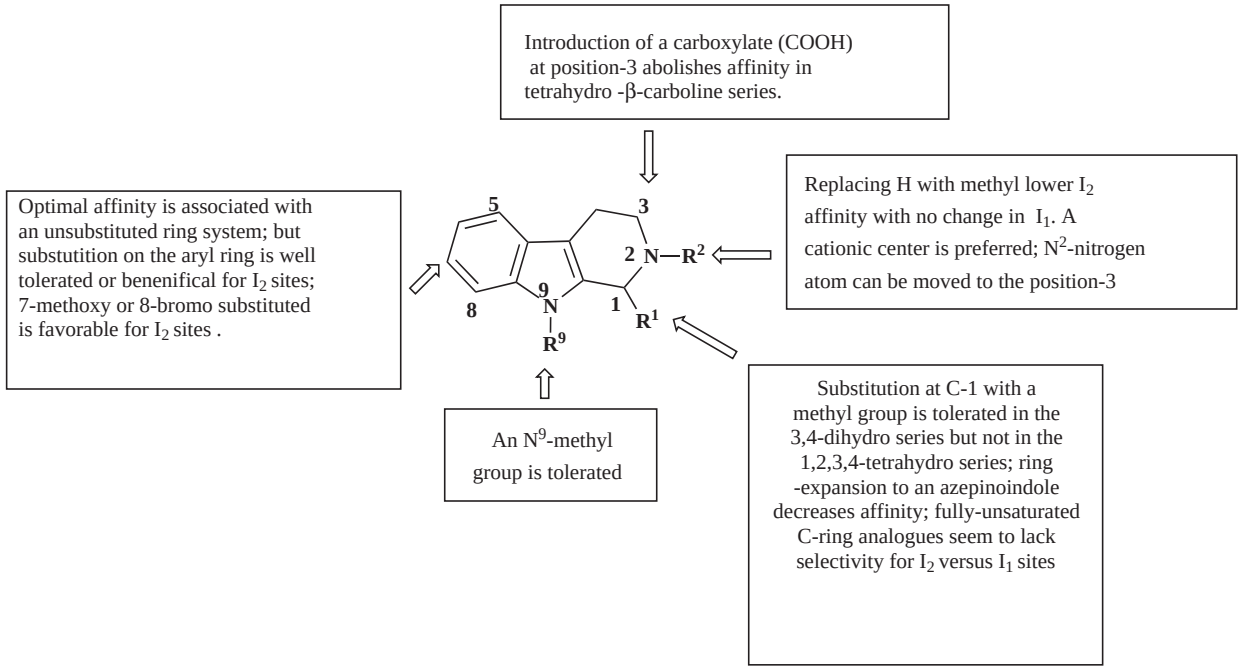


Fig. (4). The structure-activity relationships of β-carbolines binding to imidazoline sites.

HT_{1A} receptor. Not surprisingly, dopamine receptors also partly participated in the harmine (**3**) and related alkaloids-induced hypothermia effect.

Imidazoline binding sites have only recently been characterized and their pharmacological roles remain elusive. Accumulating evidence suggests that imidazoline binding sites could be important therapeutic targets [93]. In 1999, Hudson *et al*. [94] reported that certain β-carboline derivatives bound tightly and selectively to the imidazoline binding sites. Recently, harman (**2**) has been demonstrated to produce hypotension in the rat, which was attributed to activity at imidazoline (I_1) sites [95]. Studies on the synthesis of a variety of β-carboline derivatives and the evaluation of their affinities for imidazoline (I_1 and I_2) unraveled that some β-carbolines could bind with high affinity to I_2 sites and this affinity was correlated to both the planarity of the molecule and the presence of the aryl ring substituents [93]. Subsequently, a systematic structure-affinity investigation [96] was carried out to determine the influence on I_2 affinity of electron-releasing and electron-withdrawing group on the aryl portion of DHβCs and THβCs. And now structure-affinity relationships (SARs) were clearly depicted in Fig. **3**. Noticeably, norharman (**1**) has been found to bind with high affinity to imidazoline I_{2B} recepors in rat brain and liver membranes that blocks the effect of morphine withdrawl on tyrosine hydroxylase activity *in vivo* and attenuates the severity of the withdrawal reaction [97].

Of special interest of the β-carbolines such as norharman (**1**), harman (**2**) and pinoline (**10**) is that they potently stimulated insulin secretion in a glucose-dependent manner and this effect showed certain features that were consistent with the involvement of imidazoline I_3 binding sites [98]. Subsequently, Squires *et al*. [99] found that harman (**2**) might interact with ryanodine receptors to generate sustained Ca^{2+}-channels. Now it can be concluded that harman (**2**) activate at least two distinct pathways to promote insulin release, and one may be involved in binding to imidazoline I_3-receptors, while the second arises from the interaction of harman (**2**) with ryanodine receptor, leading to the generation of sustained Ca^{2+} oscillations [98-100].

In addition, harman (**2**) was found to bind to the cardiac α_1-adrenoceptors, brain 5-HT_2 receptors and cardiac 1,4-dihydropyridine binding site of L-type Ca^{2+} channels [101], and the vasorelaxant effect of harman (**2**) can be attributed to activate receptor-linked and voltage-dependent Ca^{2+} channels [101]. Interestingly, Musgrave *et al*. [102] proposed that harman (**2**) produced dose-dependent hypotension effect *via* imidazoline I_1 receptors.

β-Carbolines have shown potential antidopaminergic activity [103-104]. Low doses of harmaline (**6**) and other hallucinogenic drugs facilitate the contractile response to dopamine in isolated thoracic aorta [105-107]. Harmaline (**6**) increases dopamine-elicited hypertension in rats [108]. However, Pimpinella and Palmery [109] found that β-carbolines, such as harmine (**3**) and harmaline (**6**), dramatically facilitated dopaminergic transimission in the CNS, probably *via* interaction with Na^+-dependent membrane transporting systems. These observations were in contrasted with the antidopaminergic properties of β-carboline alkaloids, and further confirmed the facilitating dopamine-induced contractions by harmaline (**6**) in rabbit isolated aorta as well as the hypertensive effect of dopamine in rats. Subsequent or concurrent SARs analysis revealed that the methoxy group in position-7 of the β-carboline tricyclic system was responsible for increasing their potency, whereas the aromaticity of the molecule caused the opposite effect. Moreover, norharman **1** was found to induce specific, sensitive and dose-related changes in the efflux of dopamine in the nucleus accumbens of rats, and these observations suggested that several receptors mediated the effects of norharman (**1**) [109].

3. PHARMACOLOGICAL EFFECTS OF β-CARBOLINES *IN VITRO* AND *IN VIVO*

3.1. Neuropharmacological Effects of β-Carbolines *In Vivo*

The individual β-carbolines compounds have been shown to bind to different targets leading to various pharmacological effects. During the last two decades, numerous investigations had been focused on the effects of these molecules on the CNS including hallucinations, tremors, anxiety, anxiolytic, convulsions, anticonvulsant and sedation. These pharmacological effects were partially due to the interaction of the β-carboline molecules with various receptors system in the mammalian CNS, such as 5-HT receptors and BZRs.

Both harmine (**3**) and harmaline (**6**) have been demonstrated to be hallucinogenic in humans. Harmine (**3**) was inactive after oral (up to 960mg) and subcutaneous (up to 70 mg) administration, but induced some subjective effects at 35-45mg [111] and hallucinogenic effects at 150-200mg *via* intravenous administration [112]. However, harmaline (**6**) produced subjective effects in humans at about half of the dose required for harmine (**3**), and was found to be hallucinogenic at doses greater than 1mg/kg after intravenous administration. What's more, harmaline (**6**) was also orally active at a dose of 4mg/kg [112]. Tetrahydroharmine (**14**) was reported to induce subjective effects at 300mg [112]. The 6-methoxyharmalan (**15**) produced hallucinogenic effect at oral doses of 1.5mg/kg, and 6-methoxytetrahydroharman (**16**) induced modest psychoactive action at 1.5mg/kg [112]. Noticeably classic hallucinogens are thought to produce their psychoactive effects, at least in part, *via* interaction with 5-HT_2 serotonin receptors in the brain. However, so far, it has been difficult to conclude that the β-carboline alkaloids elicited hallucinogenic actions in a manner consistent with classical hallucinogens because many previous investigations demonstrated the modest interaction of β-carboline alkaloids with 5-HT receptors. It seems that the 6-methoxyl moiety contributes to the hallucinogenic effects of the compounds. Furthermore, the higher saturation in the tricyclic rings makes higher hallucinogenic effects.

Interestingly, harman (**2**) and related β-carboline alkaloids have been demonstrated to play a role in the processes of substance abuse and dependence. For instance, chronic infusion of harman (**2**) increases voluntary ethanol intake in rats [113]. Cappendijk *et al*. [114] also reported that norharman (**1**) produced prominent inhibitory effects on the naloxone-precipitated withdrawal syndrome in morphine dependent rats. Besides, high plasma levels of harman (**2**) and norharman (**1**) were found in chronic alcoholics [115] and heroin dependent humans [116-117]. Recently, Aricioglu-Kartal *et al*. [118] reported that harman (**2**) and harmine (**3**) had some beneficial effects on naloxone-precipitated

Table 3. The Neuropharmacological Effects of the β-Carbolines *In Vivo*

Compounds investigated	Neuropharmacological effects
Harmine, harmaline, 6-methoxyharmalan	Hallucinogenic [111-112]
Harman	Increase voluntary ethanol intake in rats [113]
Norharman	Inhibition of the naloxone-precipitated withdrawal syndrome in morphine dependent rats [114]
β-CCE	1. Inverse agonist [124-125] 2. Proconvulsant and convulsant in photosensitive Papio papio baboons [124-125] and in mice [128] 3. Anxiogenic in mice [126-128]
β-CCM	1. Inverse agonist [124-125] 2. Proconvulsant and convulsant in photosensitive Papio papio baboons [124-125] and in mice 3. Anxiogenic in mice [126-128] 4. Enhance animal performance [130-131]
ZK-93426	Increase of alertness and improve attention in man [132]
DMCM	1. Inverse agonist [133] 2. Prevention lethality from overdoses of pentobarbital [133]
3-HMC	Reduce of pentobarbital-induced decrements in cerebral blood flow and oxygen consumption [134]
3-Ethoxy-β-carboline	Partial inverse agonist [135-136]
ZK 93423	Agonist [139]
ZK 91296	Anticonvulsant [138]
3-Ethylamino-β-carboline	Proconvulsant activity in Papio papio baboons [79]
β-CMC	Selectively antagonize the sedative effects of diazepam [79]
FG 7142	1. Partial inverse agonist [140-143] 2. Produce a strong anxiety-like syndrome in experimental animals [140-143] and severe anxiety in man [144] 3. Improvement of performance in learning and memory tests in animals [145-147] 4. Exert stress-like effects [148]
Pinoline	Anticonvulsive [151] and antidepressant [152] in animal models

withdrawal syndrome in rats, and harmine (**3**) was more effective than harman (**2**) in reducing the sign of morphine abstinence syndrome or morphine withdrawal.

The BZRs of the mammalian CNS are known to mediate the anxiolytic, anticonvulsant, sedative/hypotic action and myorelaxant of the 1,4-benzodiazepine such as diazepam [119-121]. In the last 20 years, a wide variety of non-benzodiazepine molecules had been found to bind with high affinity to the BZ receptors, among which, a particularly well-studied class was β-carboline alkaloids. Initially, these compounds were shown to antagonize the principle pharmacological actions of the benzodiazepine [122-123]. Subsequently, many of these chemicals were found to possess intrinsic pharmacological effects that were opposite to those of the benzodiazepines. Such compounds have been termed BZR inverse agonist or antagonist. For example, 3-(ethoxycarbonyl)-β-carboline (β-CCE, **11**) and 3-(methoxycarbonyl)-β-carboline (β-CCM, **17**) were inverse agonist and of great interest since not only did they block most of benzodiazepines but actually exhibited effects opposite to those of benzodiazepines in various animal behavior models. β-CCE (**11**) and β-CCM (**27**) were, respectively, proconvulsant and convulsant in photosensitive Papio papio baboons [124-125] and in mice [128], and both compounds were anxiogenic in mice [126-128], and β-CCE (**11**) significantly increased the wakefulness of cats [129]. In addition, β-CCM (**17**) was found to enhance animal performance in three different tasks used to investigate memory and learning in three separate animal models [130-131]. Moreover, the ethyl 5-isopropoxy-4-methyl-β-carboline-3-carboxylate (ZK-93426, **19**) was shown to increase alertness and improve attention in human [132]. The BZ inverse agonist 6,7-dimethoxy-4-ethyl-3-carbomethoxy-β-carboline (DMCM, **20**) was reported to prevent lethality from overdoses of pentobarbital [133] and the 3-(hydroxymethyl)-β-carboline (3-HMC, **18**) reduced pentobarbital-induced decrements in cerebral blood flow and oxygen consumption [134]. 3-Ethoxy-β-carboline was proven to be a potent, long-lived, water-soluble partial inverse agonist [135-136]. The DMCM (**20**) retains the highly convulsive nature of β-CCM (**17**) in mice [137], while 6-(benzyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-β-carboline (ZK 93423, **21**) differed significantly in its *in vivo* activity from β-CCM (**17**) or DMCM (**20**) in that it is very similar to that of the agonist diazepam [139], and 5-(benzyloxy)-4-(methoxymethyl)-3-(ethoxycarbonyl)-β-carboline (ZK 91296, **22**) represented the only known anticonvulsant β-carboline derivative [138]. The 3-ethylamino-β-carboline showed long-lasting proconvulsant activity in Papio papio baboons, while 3-[(methoxycarbonyl)amino]-β-carboline (β-CMC, **23**) was shown in mice to selectively antagonize the sedative effects of diazepam without exhibiting convulsant, proconvulsant or anxiogenic activity by itself [79]. The 3-[(methylamino)carbonyl]-β-carboline (FG 7142, **24**), classified as a partial inverse agonist at BZRs, produced not only a strong anxiety-like syndrome in experimental animals [140-143], but also severe anxiety in human [144]. On the other hand, FG 7142 (**24**) improved performance in various learning and memory tests in animals if administered prior to training [145-147]. Recently, FG 7412 (**24**) was found to exert stress-like effects including the inhibition of locomotor exploration in post-weanling rats [148].

Most investigations have demonstrated that substitution at the position-3 of a β-carboline with an ester function was necessary for the high affinity binding to BZRs. However, Hagen *et al*. [9] reported that β-carbolines devoid of a 3-substituent could also bind to the BZRs with high affinity leading to various neuropharmalogical actions in mice. In fact, 6-(benzylamino)-3-(methoxycarbonyl)-β-carboline did not possess proconvulsant activities at the highest dose (40mg/kg) administered, while it antagonized the anticonvulsant effects of diazepam; 6-(benzylamino)-3-(hydroxymethyl)-β-carboline had proconvulsant activities but did not affect the anticonvulsant effects of diazepam; and 6-(benzylamino)-β-carboline did function as a proconvulsant and also antagonize the anticonvulsant action of diazepam [9].

In contrast to known anxiogenic β-carboline described above, pinoline (**10**) did not have any affinity for the BZR [149-150]. The binding potency of β-carbolines to the BZRs correlated well with the convulsive potential of these molecules [150]. Accordingly, pinoline (**10**) had no convulsive capacity. However, anticonvulsive [151] and antidepressant [152] properties of pinoline (**10**) have been also found in some animal models. In addition, pinoline (**10**) demonstrated a significant anxiogenic effect at pharmacological dosage [152]. These findings suggested that the neuropharmacological effect of pinoline (**10**) involved in mechanism of action other than interaction with BZRs.

3.2. Antitumor Activities of β-Carbolines *In Vitro* and *In Vivo*

Previous investigations focused on the effects of β-carboline alkaloids on the CNS. However, interests in these alkaloids were stimulated by their promising antitumor activities in the last decades. Ishida *et al*. [191] reported that harmine (**3**) and β-carboline analogues exhibited significant activities against several human tumor cell lines including three drug-resistant KB sublines with various resistance mechanisms, and α-(4-nitrobenzylidine)-harmine had a broad cytotoxicity spectrum against 1A9, KB, SaOS-2, A549, SK-MEL-2, U-87-MG and MCF-7 cells with ED50 values ranging from 0.3 to 1.2μg/ml. SAR analysis suggested that (1) introducing alkoxy substituents at C-7 led to enhanced cytotoxic activities; (2) the length of C-7 alkoxy chain affected both cytotoxicity and cell line specificity; (3) N^9-alkylated β-carboline derivatives exhibited strong cytotoxic effect; (4) C-6 brominated β-carboline derivatives showed selective cytotoxic activities; (5) N^2-alkylated β-carboline derivatives displayed specific cytotoxic activities; (6) the 3,4-dihydro-β-carboline derivatives were inactive.

Xiao *et al*. [29] reported that 3-substituted β-carboline derivatives showed cytotoxic activities against human tumor cell lines including HL-60, KB, Hela and BGC. Bis-3,4-dihydro-β-carbolines and bis-β-carbolines were synthesized and found to exhibit cytotoxic to L-1210 cells with micromolar IC_{50} [202, 205]. Additionally, 1-substituted 1,2,3,4-tetrahydro- and 3,4-dihydro-β-carboline derivatives were synthesized and evaluated [153] for the antitumor activities against murine P-388 and human KB-16, A-549 and HT-29, and all of the compounds showed significant cytotoxicities. Among them, 1-(9'-ethyl-3'-carbazole)-3,4-dihydro-β-carboline exhibited the most potent cytotoxic activities against all

tested tumor cell lines with IC_{50} <0.001μg/ml. It was worthy noted that trans-palladium (II)-harmine complex displayed remarkable cytotoxic activities against P-388, L1210 and K562 cell lines with IC_{50} 0.385, 0.385 and 0.364 μM, respectively [154]. Furthermore, platinum (II) and palladium (II) complexes of harmaline, harmalol, harmine and harman also exhibited antiproliferative activities against three tumor cells with IC_{50} varied from 0.2 to 2.0 μg/ml [207].

Our group synthesized numerous β-carboline derivatives bearing various substituents at different positions of β-carboline nucleus and evaluated their antitumor activities *in vitro* [155-157,192] and *in vivo* [155,157]. Most of the compounds showed significant cytotoxic activities *in vitro* against a panel of human tumor cell lines including non-small cell lung carcinoma (PLA-801), liver carcinoma (HepG2 and Bel-7402), gastric carcinoma (BGC-823), cervical carcinoma (HeLa) and colon carcinoma (Lovo). Some selected chemicals exhibited potent antitumor activities against mice bearing Lewis lung cancer and Sarcoma 180 with the tumor inhibition rate of over 40% [155,157]. SAR

Table 4. The Antitumor Activities of the β-Carboline Derivatives

Compounds investigated	Antitumor activities
Harmine and its β-carboline analogues	Exhibition cytotoxicities against several human tumor cell lines including 1A9, KB, SaOS-2, A549, SK-MEL-2, U-87-MG and MCF-7 cells [191]
3-Subsituted β-carboline derivatives	Exhibition cytotoxicities against several human tumor cell lines including HL-60, KB, Hela and BGC [29]
Bis-3,4-dihydro-β-carbolines and bis-β-carbolines	Display cytotoxic against L-1210 cells [202]
1-Substituted 1,2,3,4-tetrahydro- and 3,4-dihydro-β-carboline derivatives	Exhibition cytotoxicities against murine P-388 and human KB-16, A-549 and HT-29 [153]
1-Amino substituted β-carbolines	Dispay antitumor activities against the NCI 60 cell line panel [203]
Trans-palladium (II)-harmine complex	Exhibition cytotoxicities against P-388, L1210 and K562 cell lines [154]
Platinum (II) and palladium (II) complexs of β-carboline alkaloids	Exhibition antiproliferative activities against three tumor cell lines [207]
Harmine and its β-carboline analogues	1. Exhibition cytotoxicities against PLA-801, HepG2, Bel-7402, BGC-823, HeLa, Lovo [155-157] 2. Exhibition antitumor activities in mice bearing Lewis lung carcinoma and Sarcoma 180 [155-157]
Eudistomin K	1. Exhibition cytotoxicities against murine P-388 [158] 2. Exhibition antitumor activities *in vivo* against L1210, A549 and HCT-8 cell lines [158]
Eudistomidins B, C and D	Exhibition cytotoxicities against murine leukemia L1210 L5178Y cell lines [159]
Eudistomin U and its derivatives	Exhibition cytotoxicities against murine P-388 [160]
(-)-Debromoeudistomin K and its analogues	Exhibition cytotoxicities against murine leukemiaL1210, Molt/4F, MT-4 and leukemia P-388 cells [162]
Hyrtioerectines A	Exhibition cytotoxicity against HeLa cell lines [163]
Plakortamines A, B, C and D	Exhibition cytotoxicities against HCT-116 human colon tumor cell line [164]
N-methyl β-carbolinium derivatives	Exhibition cytotoxicities against four human tumor cell lines LOX, OVCAR-3, COLO-205 and MOLT-4 [165]
6-Hydroxymanzamine A, 3,4-dihydromanzamine	Exhibition cytotoxicities against L1210 and KB cell lines [166]
Manzamine A 8-Hydroxymanzamine A 8-Methoxymanzamine A	Exhibition cytotoxicities against Lovo and KB cell lines [167]
Manzamine A, Y and xestomanzamine B	Exhibition cytotoxicities against KB cells [168]
6-Deoxymanzamine X and manzamine X	Exhibition cytotoxicities against A-548, HT-29, H-116 and MS-1 cell lines [169]
Neo-Kauluamine	Exhibition cytotoxicity against human lung and colon carcinoma cells [170]
Ma'ganedin A	Exhibition cytotoxicity against murine leukemia L1210 cells [172]
Thorectandramine Fascaplysin	Exhibition cytotoxicities against MALME-3M, MCF-7, OVCAR-3 and A549 [173]
1-Substituted β-carboline alkaloids	Exhibition cytotoxicities against murine P-388 [174]
Harmine, harman and cantin-6-one alkaloids	Exhibition cytotoxicities against including breast, colon, fibrosarcoma, lung, melanoma, KB, KB-V1 and murine lymphocytic leukemia P-388 [175]
1-Methoxycanthinone, 5-methoxycanthinone	Exhibition cytotoxicities against KB, A 549, HCT-8, CAK-1, MCF-7, SK-MEL-2 [176]

analysis indicated that (1) the β-carboline structure was an important basis for the design and synthesis of new antitumor drugs; (2) appropriate substituents at position-1, 3 and 9 of β-carboline ring might play a crucial role in determining their enhanced antitumor activities; (3) the antitumor potencies of β-carboline derivatives were enhanced by the introduction of benzyl substituent into the position-2; (4) the acute toxicity of β-carboline derivatives reduced dramatically by the introduction of an appropriate substituent into the position-3 and 9; (5) the β-carboline derivatives have the potential to be used as antitumor drug leads.

In addition, 1-amino substituted β-carbolines [203] were synthesized and screened for their antitumor activities for the NCI 60 cell line panel, and 1-(N,N'-diethyl-propyl)amino-β-carboline showed the best activities with GI50 0.38μM against HOP-92 nonsmall cell lung cancer. The SAR analysis suggested that the complex polycyclic ring system in manzamine A can be substituted with simpler analogues to provide active compounds.

More recently, β-carboline amino acid ester conjugates were also found to exhibit potent cytotoxic activities against human tumor cell lines including cervical carcinoma (Hela), human breast cancer (MCF-7) and liver carcinoma (HepG2). The Lys/Arg conjugates especially demonstrated the most significant activities against human cervical carcinoma cells [194].

Marine species have been important natural sources of some promising antitumor lead compounds. Eudistomin K (**57**) exhibited potent cytotoxic activities *in vitro* against murine P-388 cells with IC_{50} value of 0.01μg/ml, and the antitumor assay *in vivo* gave a T/C of 137% at 100mg/kg, and further antitumor activities *in vivo* against L1210, A549 and HCT-8 cell lines were also reported [158]. Eudistomidins B (**45**), C (**46**) and D (**47**) showed significant cytotoxicities against murine leukemia L1210 (IC_{50} 3.4, 0.36 and 2.4 μg/ml) and L5178Y (IC_{50} 3.1, 0.42 and 1.8 μg/ml) cell lines, respectively [159]. Eudistomin U (**60**) and its derivatives were synthesized and evaluated by Dong *et al*. [160] for antitumor activities. All of the compounds exhibited cytotoxic activities *in vitro* against mouse P-388 cell strain. Eudistalbin A was shown to possess cytotoxic activity with ED50 3.2ug/ml, whereas eudistalbin B was inactive [161]. Moreover, Maarseveen *et al*. [162] found that (-)-debromoeudistomin K (**59**) and 10 structural analogues exhibited potent cytotoxic activities against murine leukemia cells (L1210), human T-lymphoblast cells (Molt/4F), human T-lymphocyte (MT-4) and P-388 leukemia cells. Especially, 5-methoxy substituted (-)-debromoeudistomin K (**59**) derivative was found to be a very potent cytotoxic compound with ID_{50} values down to 0.005ug/ml for L1210, Molt-4F, MT-4 and P-388. Furthermore, hyrtioerectines A (39) was found to possess moderate cytotoxicities against HeLa cell lines with IC_{50} 10, 5.0 and 4.5μg/ml, repectively [163], and plakortamines A (**41**), B (**42**), C (**44**) and D (**43**) displayed cytotoxic activities against HCT-116 human colon tumor cell line with IC_{50} value of 3.2, 0.62, 2.15 and 15 μM, respectively [164]. Intriguingly, three N-methyl β-carbolinium derivatives [165], isolated from marine ascidian, showed potent cytotoxic activities against four human tumor cell lines LOX (melanoma), OVCAR-3 (ovarian), COLO-205 (colon) and MOLT-4 (leukemia); 2-methyleudistomin with IC_{50} 15.0, 20.0, 19.1 and 16.6μg/ml, respectively; 2-methyleudistomin J with IC_{50} 15.1, 20.0, 15.1 and 17.5μg/ml, repectively; and 14-methyleudistomidin with IC_{50} 0.41, 0.98, 0.42 and 0.57 μg/ml, respectively.

Kobayashi *et al*. [166] reported that 6-hydroxymanzamine A (**33**) and 3,4-dihydromanzamine A (**34**) were cytotoxic against L1210 (IC_{50} 1.5 and 4.8 μg/ml, respectively) and KB cells (IC_{50} 2.5 and 0.61μg/ml, respectively) *in vitro*. Manzamine A (**27**), 8-hydroxymanzamine A (**31**) and 8-methoxymanzamine A (**32**) showed significant cytotoxicities against KB (IC_{50} 0.05, 0.30 and 0.33 μg/ml, respectively) and Lovo (IC_{50} 0.15 0.26 and 0.1μg/ml, respectively) cell

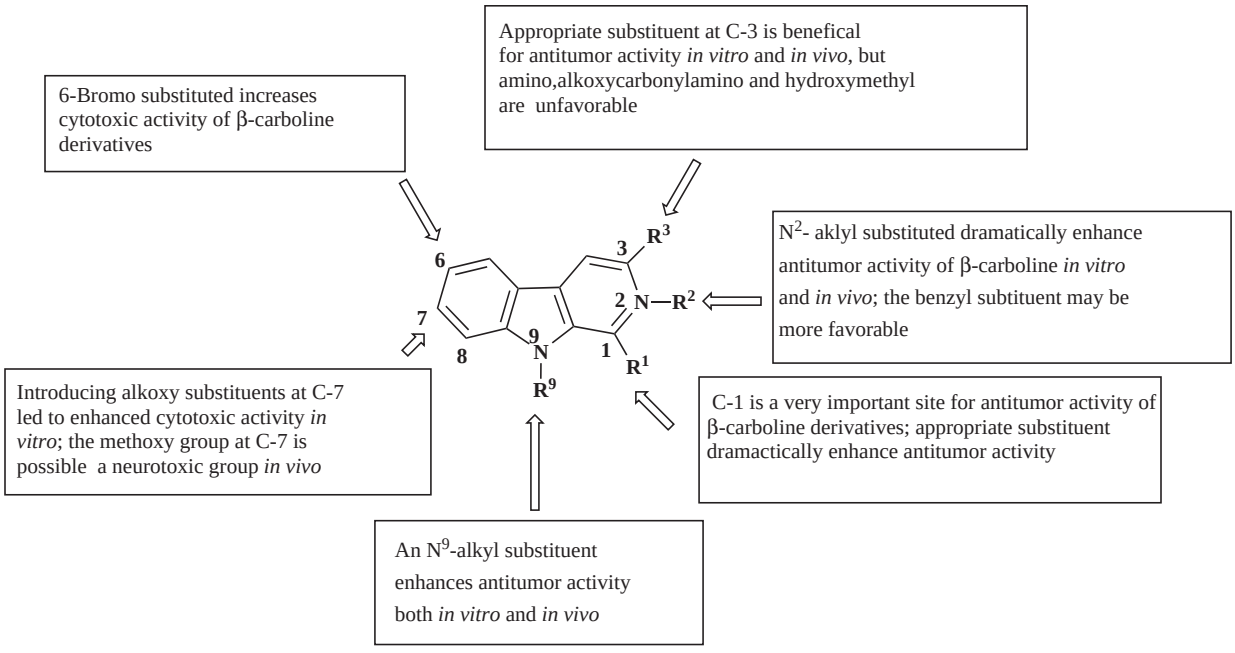


Fig. (5). The structure-activity relationships of β-carboline derivatives against tumor cells.

lines. But only manzamine A (**27**) exhibited cytotoxicity in the P-388 assay with IC_{50} 0.07 μg/ml [167]. In addition, manzamine A (**27**), Y (**30**) and xestomanzamine B (**38**) exhibited moderate cytotoxicities against KB cells with IC_{50} 7.9, 7.3 and 14.0μg/ml, respectively [168]; 6-deoxymanzamine X (**29**) and manzamine X (**28**) gave remarkable cytotoxic activities against A-548, HT-29, H-116 and MS-1 cell lines with IC_{50} in the range of 0.5-5.1 μg/ml [169]. Noticeably, neo-Kauluamine (**37**), another new manzamine dimmer, exhibited cytotoxicity with an IC_{50} 1.0μg/ml against human lung and colon carcinoma cells [170], whereas the first manzamine dimmer Kauluamine was inactive in anticancer assays [171]. Moreover, Ma'ganedin A (**26**), isolated from *Amphimedon* sponge, exhibited cytotoxicity against murine leukemia L1210 cells with IC_{50} value of 4.4μg/ml [172]. Thorectandramine (**63**), a novel hexacyclic quaternary β-carbolinium derivative from the sponge *Thorectandra sp.*, was only weakly active in MALME-3M (melanoma), MCF-7 (breast), OVCAR-3 (ovarian) and A549 (non-small lung cell cancer) with EC_{50} 27.0-55.0 μg/ml, whereas fascaplysin (**64**), another hexacyclic quaternary β-carbolinium derivative which isolated from the same species, was potently cytotoxic against four cell lines: MALME-3M (EC_{50} 0.03 μg/ml), MCF-7 (EC_{50} 0.14 μg/ml), OVCAR-3 (EC_{50} 0.16 μg/ml) and A549 (EC_{50} 0.16 μg/ml) [173]. Prinsep *et al.* [174] found that the simple β-carboline alkaloids, isolated from marine bryozoan *Cribricellina cribraria*, differed markedly in their degree of biological activity in the P-388 cytotoxicity assay [174]. 1-Vinyl-8-hydroxy-β-carboline had IC_{50} value of 100ng/ml against P-388, whereas other 1-alky substituted derivatives such as harman (**2**) and 1-ethyl-β-carboline were weakly cytotoxic. These results suggested that the vinyl group might be important for P-388 cytotoxicity.

Certain plant-derived β-carboline alkaloids are another important sources of antitumor lead compounds. Besides harmine (**3**) and harman (**2**), the cantin-6-one alkaloids, isolated from *Eurycoma longifolia*, were found to exhibit cytotoxic activities against a panel of human cancer cell types including breast, colon, fibrosarcoma, lung, melanoma, KB, KB-V1 and murine lymphocytic leukemia P-388 [175]. Recently, Xu *et al.* [176] also found that 1-methoxycanthinone (**66**) and 5-methoxycanthinone (**67**) suppressed the growth of a panel of human tumor cell lines, including epiderimoid carcinoma of the nasopharynx (KB), lung carcinoma (A 549), ileocecal carcinoma (HCT-8), renal cancer (CAK-1), breast cancer (MCF-7) and melanoma (SK-MEL-2), with IC_{50} value in the range of 2.5-20 μg/ml.

3.3. Antiviral Activities of β-Carbolines *In Vitro* and *In Vivo*

The discovery of β-carboline metabolites as potent antiviral agents has accelerated the synthetic and pharmacological studies of β-carboline derivatives. Rinehart *et al.* [177] first reported that the activities of eudistomins C (**55**), E (**56**), K (**57**) and L (**58**) against herpes simplex virus-1 (HSV-1), *in vitro*, were in the range of 25-250 ng/12.5 mm disc. Then, eudistomins D (**48**), H (**51**), I (**52**), N (**49**) and Q (**54**) were also found to exhibit modest activities against HSV-1 [178]. Besides, high activities for eudistomin K sulfoxide and the indole unsubstituted derivative eudistomin K against both HSV-1 and polio vaccine type-1 virus were also reported [179-180]. Recently, Xu *et al.* [176] reported that 1-methoxycanthin-one (**66**) was a potent anti-HIV agent with EC_{50} 0.26μg/ml and TI>39. Noticeably, platinum (II) and palladium (II) complexes of harmaline, harmalol, harmine and harman were also observed to exhibit antiviral activities against influenza virus and herpesvirus [207].

The simple β-carboline alkaloids, isolated from marine bryozoan *Cribricellina cribraria*, also displayed modest antiviral activities against HSV-1 and poliovirus grown on the BSC cell line [174]. Recently, harman (**2**) and its derivatives were found to inhibit HIV replication in H_9 lymphocyte cells, and 9-n-butyl-harmine showed potent activities with EC_{50} and therapeutic index values of 0.037 μM and 210, respectively [181].

Table 5. The Antiviral Activities of the β-Carboline Derivatives

Compounds investigated	Antiviral activities
Harmine	Antiviral activity [206]
Eudistomins C, D, E, H, I, K, L, N and Q	Antiviral activities against HSV-1 [177, 178]
Eudistomin K sulfoxide and eudistomin K	Antiviral activities against HSV-1 and polio vaccine type-1 virus [179-180]
1-Methoxycanthin-one	Antiviral activities against HIV with EC_{50} 0.26ug/ml and TI>39 [176]
Simple β-carboline derivatives	Antiviral activities against HSV-1 and poliovirus grown on the BSC cell line [174]
Platinum (II) and palladium (II) complexs of β-carboline alkaloids	Antiviral activities against influenza virus and herpesvirus [207]
Harman and its derivatives	Inhibition HIV replication in H_9 lymphocyte cells [181]
Manzamine A 8-Hydroxymanzamine A 8-Methoxymanzamine A	Antiviral activities against HSV-II [167]
(-)-Debromoeudistomin K and its 10 analogues	Inhibition replication of a number of viruses [162]
Manzamine A 8-Hydroxymanzamine A 6-Deoxymanzamine X	Anti-HIV activities against human peripheral blood mononuclear (PBM) cells [169]

Ichiba *et al.* [167] reported that manzamine A (**27**), 8-hydroxymanzamine A (**31**) and 8-methoxymanzamine A (**32**) displayed significant antiviral activities against HSV-II with MIC 0.05, 0.1 and 0.1μg/ml, repectively. Afterwards, (-)-debromoeudistomin K and its 10 structural analogues [162] were also evaluated for their inhibitory effects on the replication of a number of viruses. (-)-Debromoeudistomin K (**59**) and its enantiomer showed significant antiviral activities against influenza virus A and B in MDCK cells with MCC/MIC ratios of 10 and 13, respectively. (-)-Debromoeudistomin K and its 13-methyl as well as 10-methoxy derivatives exhibited activities against respiratory syncytial virus, vesicular stomatitis virus, Coxsackie virus B4, and polio virus-1 in HeLa cell cultures. Among them, 10-methoxy derivatives in particular had high potency with MCC/MIC ratios ranging between 13 and 67. In addition, (-)-debromoeudistomin K (**59**) and its 13-methyl as well as 10-methoxy derivatives showed the most promising activitities against various HSV-1 and HSV-2 strains in PRK cell cultures with MCC/MIC values ranging from 19 to 125, 13 to 57 and 45 to 294, respectively. The SARs studies indicated that the significant antiviral activity is depended upon both natural stereochemistry at both C (1) and C (13b) and the presence of the C (1) $-NH_2$ substituent. Recently, manzamine A (**27**), 8-hydroxymanzamine A (**31**) and 6-deoxymanzamine X (**29**) were also found to possess anti-HIV activities against human peripheral blood mononuclear (PBM) cells with median effective concentrations (EC_{50}) 0.59, 4.2 and 1.6 μM, respectively [169].

3.4. Antimicrobial Activities of β-Carbolines *In Vitro*

Currently, only a few studies have been published on the antimicrobial activities of β-carboline alkaloids. The eudistomins H (**51**), I (**52**), O (**50**) and P (**53**) exhibited modest antimicrobial activities against *Saccharomyces cerevisiae*, and the eudistomins D (**48**), I (**52**), N (**49**), O (**50**), P (**53**) and Q (**54**) showed moderate activities against *Bacillus subtilis*, a gram-positive bacterium [178]. Ma'ganedin A (**26**) was found to display antibacterial activity against *Sarcina lutea* (MIC 2.8μg/ml), *Bacillus subtilis* (2.8 μg/ml) and *Corynebaceterium xerosis* (5.7μg/ml) [172]. Gesashidine A (**40**) showed antibacterial activities against *Micrococcus luteus* (MIC 16.6 μg/ml) [182].

In addition, certain simple β-carboline alkaloids, isolated from marine bryozoan *Cribricellina cribraria*, showed antimicrobial activities against two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Eschericbia coli*), a Gram-positive bacterium (*Bacillus subtilis*) and three fungi (*Candida albicans*, *Trichophyton mentagrophytes*, and *Cladisporum resinae*) [174]. Schupp *et al.* [183] reported that eudistomins X (**62**) exhibited antibiotic activity toward *Bacillus subtilis*, *Staphyloccocus aureus* and *Escherichia coli*, and were also fungicidal agaist *Candida albicans* in an agar diffusion assay. Eudistomins W (**61**) was selectively active against *Candida albicans* but showed no antibacterial activities.

Most manzamines were active against *Mycobacterium tuberculosis* ($H_{37}Rv$) with MICs <12.5 μg/ml. The (+)-8-hydroxymanzamine A showed the most significant antibacterial activities with MIC of 0.91μg/ml [169]. Moreover, 6-hydroxymanzamine A (**33**) and 3,4-dihydromanzamine A (**34**) showed antibacterial activities against a Gram-positive bacterium, *Sarcina lutea* (MIC value, 1.25 and 4.0 μg/ml) [166].

3.5. Antiparasitic Activities of β-Carbolines *In Vitro* and *In Vivo*

In the last two decades, the antiparasitic activities of β-carbolines have attracted increasing attention. Harmaline (**9**) exhibited significant antiparasitic activities against *Leishmania mexicana amazonensis* both *in vitro* and *in vivo* [184] and displayed antileishmanial activity toward the intracellular amastigote form of Leishmania [204]. While harmine (3) and harman (**2**) inhibited both extracellular flagellated promastigote and intracellular amastigote forms of Leishmania [204]. β-Carboline-3-carboxaldehyde thiosemicarbazone (69) and 3-acetyl-β-carboline thiosemicarbazone (70) were also found to be lethal to promastigotes of Leishimania donovani, and 50% inhibition at concentration of 5.0 and 2.5 μM, respectively, while irreversible growth inhibition was achieved at 40 and 17.5μM, repectively, and β-CCM (**27**) was practi-

Table 6. The Antimicrobial Activities of the β-Carboline Derivatives

Compounds investigated	Antimicrobial activities
Eudistomins H, I, O, P	Antimicrobial activities against *Saccharomyces cerevisiae* [178]
Eudistomins D, I, N, O, P, Q	Antimicrobial activities against *Bacillus subtilis* [178]
Ma'ganedin A	Antibacterial activity against *Sarcina lutea* (MIC 2.8μg/ml), *Bacillus subtilis* (2.8 μg/ml) and *Corynebaceterium xerosis* (5.7μg/ml) [172]
Gesashidine A	Antibacterial activity against *Micrococcus luteus* (MIC 16.6 μg/ml) [182]
Simple β-carboline alkaloids	Antimicrobial activities against *Pseudomonas aeruginosa*, *Eschericbia coli*, *Bacillus subtilis*, *Candida albicans*, *Trichophyton mentagrophytes*, and *Cladisporum resinae*) [174]
Eudistomins X	1. Antibiotic activity toward *Bacillus subtilis*, *Staphyloccocus aureus* and *Escherichia coli* [183]; 2. Fungicidal against *Candida albicans* [183]
Eudistomins W	Fungicidal agaist *Candida albicans* [183]
Manzamines	Antibacterial activity against *Mycobacterium tuberculosis* ($H_{37}Rv$) with MICs <12.5 μg/ml [169]
6-Hydroxymanzamine A, 3,4-dihydromanzamine A	Antibacterial activity against a Gram-positive bacterium, *Sarcina lutea* (MIC, 1.25 and 4.0 μg/ml) [166]

cally inactive up to 50 μM [185]. In additon, harmine (**3**) and related β-carboline alkaloids [186] all exhibited trypanosomicidal activities *in vitro* against *Trypanosoma cruzi* epimastigotes belonging to two different strains (Tulahuen and LQ) showing different sensitivity to nifurtimox, but only harmine (**3**) has significant activities at the concentration of 100μM. Harmine (**3**) inhibited growth of the Tulahuen strain by 90% by day 10, and by 88% by day 7 in the LQ strain; harman (**2**) showed an inhibition of 68% by day 10 in the Tulahuen strain and of 67% by day 7 in the LQ strain; and harmaline (**9**), norharman (**1**) and harmol (**20**) displayed very similar and moderate activities in the Tulahuen strain with inhibition of cultures by 45-47% after 10 days, whereas harmalol (**10**), THβC (**4**) and MTHβC (**5**) were weakly active. Recently, a series of 1-amino substituted β-carbolines were synthesized and screened against the parasites T. cruzi (tulahuen C4 strain), P. falciparum (K1strain), L. donovani (MHOM-ET-67/L84 strain) and T.b. rhodesiense (STIB 900 strain) by the World Health Organization (WHO) [203], all compounds were observed to exhibit significant antiparasitic activities.

Certain 1,3-disubstituted β-carboline derivatives [187] exhibited either more than 90% microfilaricidal or macrofilaricidal activities or sterilization of female worms *in vivo* against *Acanthoeilonema viteae*. Among them, methyl 1-(4-methylphenyl)-β-carboline-3-carboxylate showed the highest adulticidal activity and methyl 1-(2-chlorophenyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylate displayed the highest microfilaricidal action against *Acanthoeilonema viteae* at 50mg/kg×5 days *via* intraperitonneal (i. p.) route. 1-(4-chlorophenyl)-3-hydroxymethyl-β-carboline exhibited the highest activity against Litomosoides carinii at 30mg/kg×5 days (i. p.) and against Brugia malayi at 50mg/kg×5 days (i. p.) or at 200mg/kg×5 days through (p. o) route. The SARs studies showed that the presence of a carbomethoxy at position-3 and an aryl substituent at position-1 in β-carboline nucleus effectively enhanced the antifilarial activities particularly against *A. viteae*.

Manzamine A (**27**) and its hydroxy derivatives, (-)-8-hydroxymanzamine A, were found to be active against the asexual erythrocytic stages of *Plasmodium beighei* [188]. More than 90% of the asexual erythrocytic stages of *P. beighei* were inhibited after a single intraperitoneal injection (50 or 100 μM) of manzamine A (**27**) and (-)-8-hydroxymanzamine A into infected mice. Especially, most infected mice treated with manzamine A (**27**) were able to survive for a longer period of time carrying fulminating recurrent parasitemia. Two mice, treated with 100 μM/ml of manzamine A (**27**) per kg, were able to recover and clear the parasitemia completely. Oral administration of manzamine A (**27**) also produced significant reductions in parasitemia. Interestingly, manzamine F, a ketone analog of manzamine A (**27**), was found to be inactive. El Sayed *et al.* [170] also reported that the new enantiomers of 8-hydroxymanzamine A (ent-8-hydroxymanzamine A, **35**) and manzamine F (ent-manzamine F, **36**), isolated from Indo-Pacific sponge, together with manzamine A (**27**), exhibited significant activities against *T. gondii*. Ent-manzamine F (**36**) showed a 37% inhibition of the parasite at 10 μM concentration without toxicity to host; while manzamine A (**27**) displayed 70% inhibition of the parasite at 0.1μM concentration without host cells toxicity, and the activity was significantly increased at concentrations of 1 and 10 μM accompanied by an increase in the toxicity for the host cells. The ent-8-hydroxymanzamine A (**35**) inhibited 71% of the parasite at 1μM, along with 38% inhibition of the host cells. A daily i. p. dose of 8mg/kg of manzamine A (**27**), for 8 consecutive days, beginning on day 1 following the infection prolonged the survival of SW mice to 20 days, as compared with 16 days for the untreated control. In addition, most of the manzamines with exception neo-kauluamine (**37**), induced 98-99% inhibition of *Mycobacterium tuberculosis* ($H_{37}Rv$) with

Table 7. The Antiparasitic Activities of the β-Carboline Derivatives

Compounds investigated	Antiparasitic activities
Harmine	Antileishmanial against the promastigote and amastigotes of *Leishmania* [204]
Harman	Antileishmanial against the promastigote and amastigotes of *Leishmania* [204]
Harmaline	Antiparasitic against *Leishmania mexicana amazonensis* [184] Antileishmanial against the amastigote stage of *Leishmania* [204]
β-Carboline-3-carboxaldehyde thiosemicarbazone) 3-Acetyl-β-carboline thiosemicarbazone	Lethal to promastigotes of *Leishmania donovani* [185]
Harmine and related β-carboline alkaloids	Trypanosomicidal *in vitro* against Trypanosoma cruzi epimastigotes [186]
1,3-Disubsitituted β-carboline alkaloids	Microfilaricidal or macrofilaricidal activity or sterilization of female worms *in vivo* against *Acanthoeilonema viteae* [187]
1-Amino substituted β-carbolines	Antiparasitic against *T. cruzi, P. falciparum, L. donovani* and *T.b. rhodesiense* [203]
Manzamine A (-)-8-Hydroxymanzamine A	Antiparasitic against the asexual erythrocytic stages of *Plasmodium beighei* [188]
Ent-8-hydroxymanzamine A Ent-manzamine F	Antiparasitic against *T. gondii* [170] and *P. berghei* [189]
Manzamines	Antimalarial against *Plasmodium falciparum* and *Leishmania donovani* [169]
β-Carboline derivatives	Antimalarial against three *Plasmodium flaciparum* clones, W2, D6 and TM91C235 [190]

MIC <12.5 μg/ml, especially, manzamine A (**27**), E and ent-8-hydroxymanzamine A (**35**) exhibited MIC endpoint of 1.56, 3.13 and 3.13 μg/ml, respectively. Moreover, *in vivo* assay against *Plasmodium berghei*, a single intraperitoneal (i. p.) dose of 100 μM/kg ent-8-hydroxymanzamine A (**35**), ent-manzamine F (**36**) and neo-kauluamine (**37**) to mice displayed no apparent toxicity [189], ent-8-hydroxymanzamine A (**35**) and neo-kauluamine (**37**) efficiently reduced parasitemia with an increase in the average survival days of *Plasmodium berghei* mice (9-12 days), as compared with untreated controls (2-3 days), mice treated with misinin (2 days) and chloroquine (6 days). Noticeably, three 50 uM/kg i. p. dose of ent-8-hydroxymanzamine A (**35**) were found to be curative and totally cleared the parasite, and two oral doses (100μM/kg) provided a remarkable reduction of parasitemia.

Recently, the antimalarial activities of manzamines against malaria parasite *Plasmodium falciparum* [169,195] and *Leishmania donovani* [169], the causative agent for visceral leishmaniasis, were also reported. Furthermore, some β-carboline derivatives, isolated from *Eurycoma longifolia*, were found to be effectively antimalarial against three *Plasmodium flaciparum* clones, W2, D6 and TM91C235 [190].

3.6. Antithrombotic Activities of β-Carbolines *In Vitro* and *In Vivo*

Only a few investigations have been published on the antithrombotic activities of β-carboline derivatives. Tang *et al*. [197-199] first reported that perlolyrine (**71**) and its analogues exhibited potent anti-aggregation activities *in vitro* and antithrombotic activities *in vivo*. SAR analysis suggested that (1) the β-carboline structure might be an important basis for their anthrombotic activities; (2) the substituents at position-1 of β-carboline might be their pharmacophore or pharmacokinetics group; (3) the antithrombotic activities of β-carbolines were enhanced by the introduction of appropriate substituents into position-1 of β-carboline ring. Subsequently, Lin *et al*. [200] found that the linkers of tetrapeptides (RGDS, RGDV, RGDF) and 3S-1,2,3,4-tetrahydro-β-carboline-3-carboxyl acid by linking the carboxyl group of β-carbolines with the amino group of peptides through amidation reaction, exhibited remarkable anti-aggregation and anti-adhesion activities *in vitro* and antithrombotic activities *in vivo*. The antithrombotic potencies of tetrapeptides were obviously enhanced by the introduction of 3S-1,2,3,4-tetrahydro-β-carboline-3-carboxyl acid group into their alpha amino group of tetrapeptides. Recently, a series of novel dipeptide analogues by incorportating tetrahydro-β-carboline-3-carboxylic acid skeleton as an amino acid surrogate were reported to display potent dual antiaggregation activities in both of ADP- and PAF-induced platelet aggregation assay *in vitro*, and these dipeptide analogues were observed to show the dose-depedent antithrombotic effect *in vivo* rat arterial thrombosis model [201]. SAR analysis indicated that (1) 3S-1,2,3,4-tetrahydro-β-carboline-3-carboxyl acid was an important scaffold for their antithrombotic activities; (2) the nature of the amino acid residues introduced into position-3 of β-carboline ring significantly affected their antiplatelet and antithrombotic activities; (3) the polarity, charge, molecular size, and the spatial arrangement of these β-carboline might be the key factors in influencing their biological activities.

4. CONCLUSIONS

Increasing evidences have substantially accumulated to support that β-carboline and related derivatives widely occurred in nature, especially in various tissues and body fluids of human. And human beings are sufficiently exposed to various β-carboline alkaloids, which are both present in plants used for the preparation of hallucinogenic materials and medicinal drugs, and in tobacco smoke and well-cooked food [10, 186, 241-243]. In addition, previous investigations indicated that human beings can endogenously form various β-carboline alkaloids, such as norharman and harman. The proposed biosynthesis pathways of these "endogenous alkaloids" in human body fluids and tissues have attracted much concern because of their possible influence on the central nervous function in the last two decades. However, so far, it has been debated whether substantial amounts of them are derived from diet or physiologically [240].

Undoubtedly, the β-carbolines had extensive biochemical activities and multiple pharmacological effects. Individual compounds might selectively interact with specific targets so as to lead to a variety of pharmacological actions *in vitro* and *in vivo*. Therefore, the β-carboline alkaloids might be a particularly promising lead compounds for discovering and developing novel clinical drugs. Taking all those reports together, we might conclude that (1) the β-carboline structure was an important basis for the design and synthesis of novel clinical drugs; (2) various substituents at different positions of β-carboline ring system might play a crucial role in determining their multiple pharmacological function; (3) the substituents at position-1, 2, 3 and 9 of β-carboline might be important pharmacophore for their antitumor activities; while the substituents at position-3 might be vital for their exhibiting various neuropharmacological effect; the nature of substituents at position-1 and 3 might contritute to their antiparasitic activities or antithrombotic activities.

However, it was also worthy to note that certain β-carbolines were very dangerous. Harman (**2**) and norharman (**1**) were comutagens or precursors of mutagens; TaClo (**21**), TaBro (**68**) and N-methylated β-carboline derivatives were potent endogenous neurotoxins; and N-nitroso derivatives of β-carboline and APNH (**25**) derivatives were endogenous mutagens and carcinogens. On the other hand, human are continuously exposed to endogenous and exogenous β-carboline alkaloids. Thus, a rising need, the study on how to deal with them and how to utilize them, especially, their biological and pharmacological activities, should be brought into our mind instantly to reduce their potential risk and to develop new drugs. Moreover, further studies *in vivo* with respect to possible actions on human health are urgently required.

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