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Mini-review

Norharmane as a potential chemical entity for development of anticancer drugs

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ABSTRACT

Cancer is a leading cause of death generally, and to overcome this problem the introduction of a new drug developing is a continuous endeavour. An alkaloid, norharmane and its derivatives, which have anticancer activities, widely distributed in several living and synthetic chemical sources. Herewith, the suggested mechanisms of organic reactions and synthetic approaches of norharmane available so far were considered. Active sites of norharmane nucleus positions, C-1, C-3, and N-9, were used for developing new molecules and based on structure activity relationship (SAR), those have been seen with anticancer activities. This review summarizes on chemistry of synthetic strategies of norharmane derivatives, which may provide a framework to design a novel anticancer drug, in future.

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1. Introduction

Globally cancer is the second leading cause of morbidity and death, and nearly 1 in 6 deaths is due to cancer [1], In India, for instance, cancer is emerging as a major public health concern. The age-standardized prevalence of cancer is estimated to be 97 per 100,000 persons with greater prevalence in urban areas [2]. FDA recommended anticancer drugs are mostly unaffordable, while plant-based anticancer agents are limited or have not been established yet, viz., paclitaxel, curcumin, strigolactones and cannabinoids [3].

Since time immemorial, natural products have been employed to treat several diseases traditionally as ethnomedicines. Particularly, plant products have popularity in most countries for example, China, India, Japan, Korea, Australia, Africa and Russia [4] as indigenous concoctions of crude phyto-extracts, have been ameliorating diseases, without any scientific verification. Furthermore, several pure phytochemicals have been established scientifically in mainstream medicinal system today, for example alkaloids viz, quinine, reserpine, morphine, taxol and a few more [5]. Indeed, alkaloids are often used in drug development cascades. The isolation of the alkaloid, norharmane was followed by chemical modifications as for sulpha drugs [6]. The synthesis of doppelgängers to isolated pure compounds is not possible, nor could be obtained from natural source en mass to meet the demand. Eventually partial synthesis of a drugable molecule, or isolation from any of several natural sources was followed by side chain modifications are preferred specifically suiting to a disease-target.

IUPAC nomenclature of norharmane nucleus (Fig. 1) is 9*H*-pyrido[3,4-*b*]indole, which is a chemically β-carboline derivative with a fusion of two moieties of indole and pyridine. Herein, synthetic/semi-synthetic strategies in the preparation of norharmane and its derivatives were considered to yield several anticancer molecules, which have been reported and discussed with several functionality substituted in norharmane nucleus and its structure activity relationship (SAR) activities.

Similar examples are plentiful with cyanocompounds from the cyanobacterium (blue-green alga), *Lyngbya* sp. [7]. The structure of norharmane has lent to chemical modifications at several positions for development as drugs [8].

1.1. Sources of norharmane

Norharmane was first reported from a higher plant, *Tribulus terrestris* (family, Zygophyllaceae). Moreover, it was also reported from animals, *Reticulitermes termites,* marine sponge,

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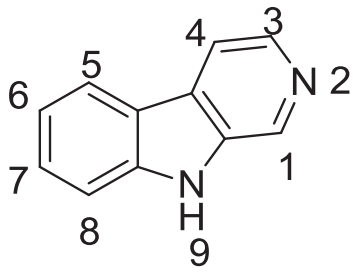
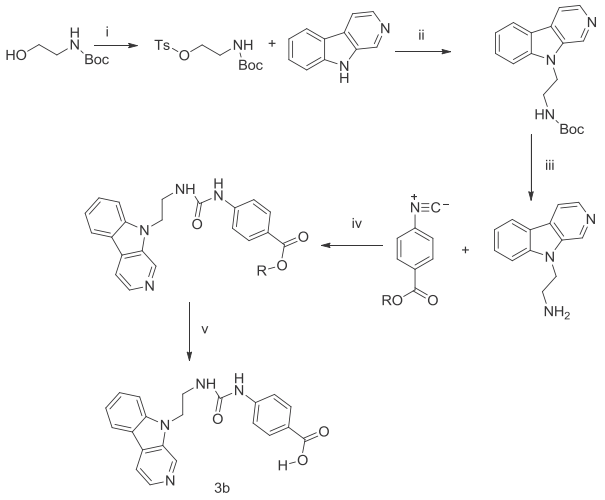


Fig. 1. Structure of norharmane nucleus.

Hymeniacidone perleve and several cyanobacteria (Table 1). Norharmane has several biological efficacies especially in eliminating cancer cells for which, it is a popular candidate with among medicinal chemists [9]. The derivative of norharmane, Normelinonine F (2-methyl-9H-pyrido[3,4-*b*]indole or 2-methyl-norharmanium) and Melinonine F (1,2-dimethyl-9H-pyrido[3,4-*b*]indole or 2-methyl-harmanium) were found in the root bark of *Strychnos usambarensis* [10].



Reactions : (i) TsCl, Py, RT, 12h; (ii) NaH, DMF, 450C, 16h; (iii) TFA, DCM, RT, 5h; (iv) BTC, Et3N, DCM, RT-Reflux, 2h; (v) NaOH, H2O/C_2H_5OH,100^{0}C, 2h

Scheme 1. N-substituted norharmane.

2. Synthesis and anticancer activity of norharmane derivatives

2.1. Synthesis of N-substituted norharmane

In Scheme 1, a series of 9-substituted derivatives of norharmane were synthesized and the end compound, 3b, 4-(3-(2-(9H-pyrido [3,4-b] indol-9-yl) ethyl) ureido) benzoic acid synthesized by the treatment of an intermediate precursor 2-(9H-pyrido[3,4-*b*]indol-9-yl) ethanamine and derivative of isocyanate in the presence of dichloromethane under refluxed condition and the product was subjected to hydrolysis to get the desired ester, 3b. The SAR of all the prepared compounds having the β-carboline moiety linked with different substitution such as, (i) *N*-benzylpropan-1-amine, (ii) 1-phenyl-3-propylurea and (iii) *N*-benzyl propionamide at N-9 position ended at the synthesis of 3b, which inhibited resulted 35 and 52% inhibition levels at 30 and 100 μM, respectively. These results indicated that due to presence of bridge linking 4-caroboxy, n-phenyl -3-propylurea with norharmane inhibitory activity against sirt5 [22].

2.2. Synthesis of 1,3-disubstituted norharmane possess oxindolin-2-one

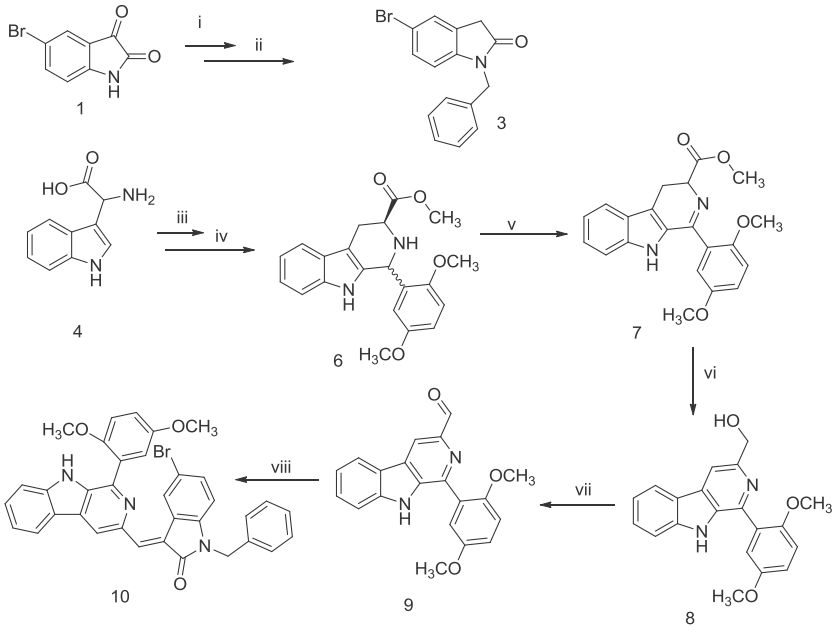
In Scheme-2, the series of β-carboline-indolinone hybrids were synthesized, with several steps, the final step of synthesized compound 10, E−1-benzyl-5-bromo-3-((1-(2,5-dimethoxyphenyl)-9H-pyrido[3,4-b] indole-3-yl) methylene) indolin-2-one was obtained by the condensation reaction of intermediate 1-benzyl-5-bromoindolin-2-one with 1-(2,5-dimehoxyphenyl)-9H-pyrido [3,4-b] indole-3-carbaldehyde. In presence of piperidine in ethanol under reflux for 2−4hr. The potent compound 10 exhibited anticancer activity against selected cell lines viz., HCT-15/116, A549 and MCF-7. with significant IC_{50} values against HCT-15 only [23].

2.3. Synthesis of 1,3 substituted norharmane bearing 1,2,3-triazole

In Scheme-3, a series of C-3 were linked to 1,2,3-triazolo-β-carboline and the generated hybrids synthesized by substituted 9H-pyrido indolyl azides with different substituted alkynes in presence of copper sulphate and sodium ascorbate. In these

Table 1
Isolated of norharmane moiety from different sources.

Sl No	Name of organism	Sources	Family	Reference
1	*Anabaena cylindrica*	Cyanobacterium	Hormogoneae	[11]
2	*Anabaena inaequalis*	Cyanobacterium	Hormogoneae	[11]
3	*Anabaena oryzae*	Cyanobacterium	Hormogoneae	[15]
4	*Anabaenopsis siamensis*	Cyanobacterium	Hormogoneae	[11]
5	*Chroococcus minutus*	Cyanobacterium	Chroococcaceae	[11]
6	*Chrysophyllurn lacourtianum*	Angiosperm	Sapotaceae	[9]
7	*Festuca arundinacea*	Angiosperm	Poaceae	[9]
8	*Geitlerinema carotinosum*	Cyanobacterium	Coleofasciculaceae	[15]
9	*Lolium perenne*	Angiosperm	Poaceae	[9]
10	*Nocardia* sp.	Actinobacterium	Norcardiaceae	[16]
11	*Nodularia harveyana*	Cyanobacterium	Aphanizomenonaceae	[11,14]
12	*Nostoc linckia*	Cyanobacterium	Nostocaceae	[15]
13	*Nostoc carneum*	Cyanobacterium	Nostocaceae	[11]
14	*Reticulitermes termites*	Arthropoda	Rhinotermitidae	[12]
15	*Synechocystis aquatilis*	Cyanobacterium	Chroococcaceae	[13]
16	*Tribulus terrestris*	Angiosperm	Zygophyllaceae	[17]
17	*Strychnos bamhartiana*	Angiosperm	Loganiaceae	[18]
18	*Evodiae fructus*	Angiosperm	Rutaceae	[19]
19	*Coffea* sp.		Rubiaceae	[20,21]



Reaction: (i) K_2CO_3, DMF, C_6H_5Br, Overnight; (ii) NH_2NH_2, H_2O,100^{0}C, Reflux, 5h; (iii) $SOCL_2$MeOH, RT; (iv) 5% TFA/DCM, 2,5-dimethoxybenzaldehyde, RT, 8h; (v) $KMnO_4$, THF, RT, 8h; (vi) $LiAlH_4$, THF, 0^{0}C, 2h; (vii) MnO_2, DCM, RT, 6h; (viii) Piperidine, EtOH, Reflux, 2-4h.

Scheme 2. 1,3-disubstituted norharmane possess oxindolin-2-one.

reactions, cupper (I)-catalyzed azide-alkyne cycloaddition was performed to afford compounds bearing triazole motif. Both compounds 3 and 4 were synthesized by substitution of methoxy 9H-pyrido indolyl azides with 5-methyl-2-(prop-2-yn-1-yl) isoindoline-1,3-dione and 2-(prop-2-yn-1-yl)-1H-benzo[de]isoquinoline-1,3(2H)-Dione respectively. The compound, 3 with a trimethoxy phenyl at C1-position and 1,2,3-triazolo methyl phthalimide at C3-position, compound 4 with dimethoxy phenyl substituent at C1-position and 1,2,3-triazolo naphthalimide at C3-position exhibited the HGC-27 cell line, among which, the compound 3 had the highest cytotoxic activity with the IC_{50} as 5.44 ± 0.58, μM [24].

2.4. Synthesis of 1, 3-disubstituted norharmane with salicylamide

In Scheme-4, norharmane salicylic conjugates were synthesized by the mixture of an intermediate, methyl 1-methyl-9H-pyrido [3, 4-b] indole-3-carboxylate 4 and 1, 4 diamino butane in alcohol to obtain the product *N*-(2-aminoethyl)-1-methyl-9H-pyrido [3,4-b] indole-3-carboxamide 5, which was treated with acetylsalicylic acid in the presence of ethyl chloroformate and n-methyl morpholine; the product 2-(((1-methyl-9H-pyrido[3,4-*b*]indole-3-carboxamido)butyl)carbamoyl)phenyl acetate 6 and finally the ester product on hydrolysed gave the compound, *N*-((2-hydroxybenzamido)butyl)-1-methyl-9H-pyrido[3,4-*b*]indole-3-carboxamide 7. The compound 7, the norharmane salicylamide conjugate had antiproliferative activity on human hepatocellular carcinoma cells (SMMC-7721 and Hep G2), human colon cancer cell lines (HCT116), human bladder carcinoma cells (EJ), and human lung cancer cells (H460) *in vitro*. This compound was reported inhibiting the proliferation of liver cancer SMMC-7721 cells and exhibited anti-proliferation activity on SMMC-7721 cells with IC_{50} value of 6.97 μM [25].

2.5. Synthesis of 3, N-substituted of norharmane

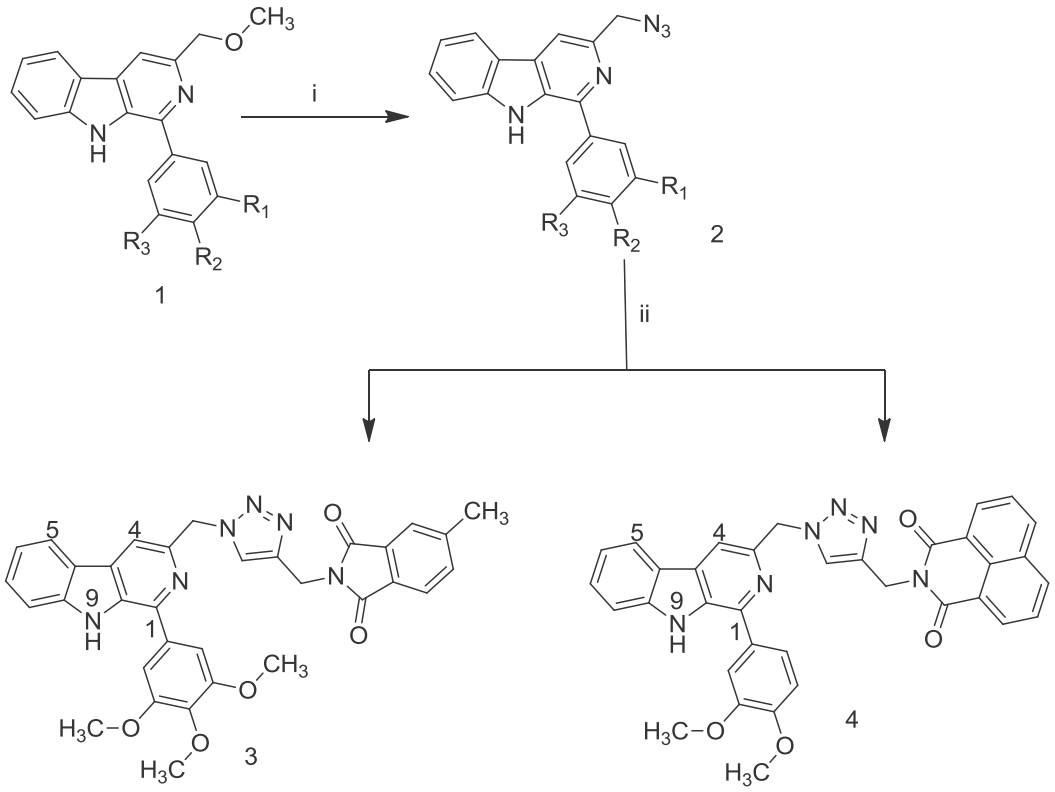
In Scheme 5, the compound 5 was prepared by reduction of individual mixture of 9-phenanthrenyl, β-carboline 3-ethylcarboxylate and lithium aluminum hydride (LAH). The compounds 6a, 6b and 6c were prepared from 1-methyl 9-substiuted naphthyl β-carboline 3-ethylcarboxylate by treatment with methyl magnesium bromide (Grignard's regent), whereas the compound 6d was developed by a reduction with LAH of 9-phenanthrenyl, 1-methyl β-carboline 3-ethylcarboxylate. Compounds 5, 6a, 6b, 6c and 6d displayed remarkable activity, with IC_{50} values of 0.75, 0.91, 1.00, 1.13 and 2.54 μM against cancer cell lines, the HL-60, SMMC-7721, A-549, MCF-7 and SW480, respectively [26].

2.6. Synthesis of 1-methyl, 2,3,N-trisubstituted norharmane

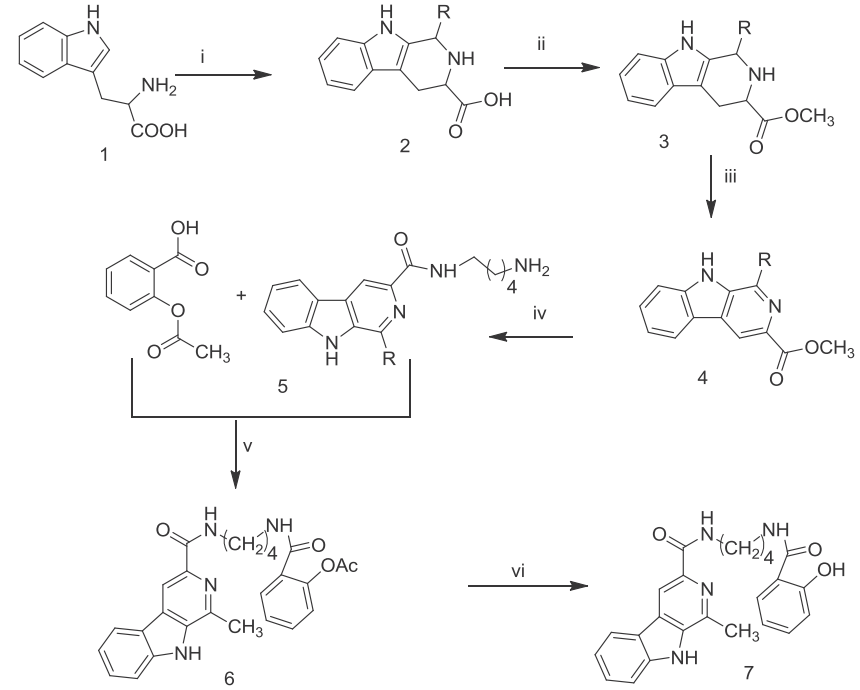
In Scheme-6, an intermediate compound, 1,2,3,4-tetrahydro carboline 3-carboxrlic acid was mixed with thionyl chloride in dry ethanol, which caused conversion to ethyl carboxylate 2 that was refluxed with toluene in presence of the catalyst manganese dioxide; on oxidization it produced final obtained compound, 9-(2-methoxybenzyl)-β-carboline-3-carboxylic acid 3. The compound 3 inhibited the growth of HL-60 cells by inducing apoptosis at the concentration, 4.0 μM [27].

2.7. Synthesis of 1-methyl, 2,3,N-trisubstituted norharmane

In Scheme-7, 1-methyl- β-carboline of benzyl groups were disubstituted at N^9 and N^2 positions of β-carboline in an intermediate step of the compound, with the presence of sodium hydride in anhydrous dimethylformide (DMF) to produce 2a (2, 9-dibenzyl-1-methyl-9H-pyrido[3,4-*b*]indol-2-ium bromide), whereas the compound 3b (2,9-dibenzyl-3-(ethoxycarbonyl)-1-methyl-9H-pyrido

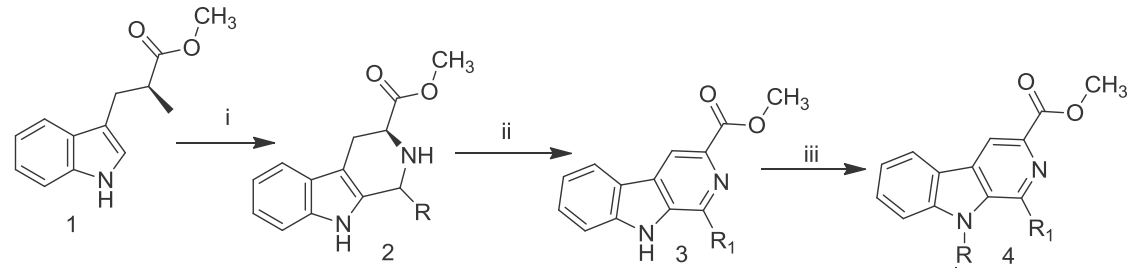


Scheme 3. 1,3 substituted norharmane bearing 1,2,3-triazole.



Reactions: (i) H^+ or OH^-, RCHO, reflux, 2-4h; (ii) $SOCl_2$, MeOH, 0^0C, 1h and then reflux 6h; (iii) $KMnO_4$, DMF, reflux,6h; (iv) Butane 1,4- diamine, EtOH, reflux, 3-5h; (v) a. N-methylmorpholine, ethyl chloroformate, THF,0^0C,1h ; b.Et_3N, THF,0^0C,1-3h

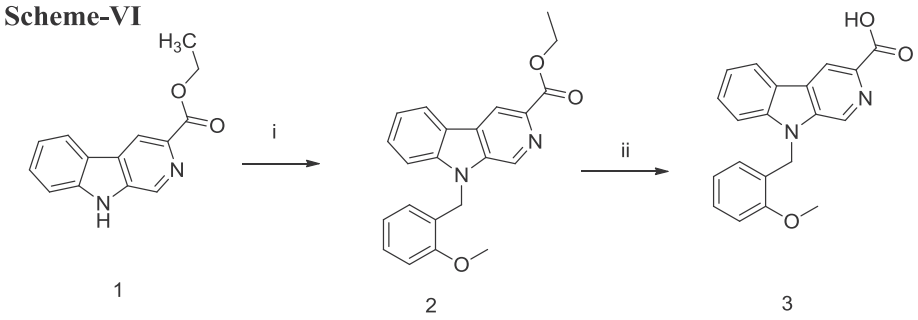
Scheme 4. 1, 3-disubstituted norharmane possess salicylamide.



Compd.	R	R_1	R_2	R_3
5	----	Phenanthryl	H	H
6a	CH_3	Napthyl	H	H
6b	CH_3	Napthyl	CH_3	CH_3
6c	CH_3	Methoxy-napthyl-	CH_3	CH_3
6d	CH_3	Phenanthryl	H	H

Reaction: (i) Aldehyde, CH_2Cl_2, Molecular sieve, RT, 24h to a week, then TFA, toluene, 60^0C, 24h; (ii) DDQ, CH_2Cl_2, RT, 0.5h; (iii) CH_3l/CH_3CH_2Br, K_2CO_3, DMF, RT, 12h; (iv) Compd 5. $LiAlH_4$, THF,RT, 1h;Compd 6a-6d. CH_3MgBr,THF, RT 6-10h

Scheme 5. 3, N-substituted of norharmane.



Reactions: (i) 2 methoxy benzyl halide, KOH, THF; (ii) 50%C_2H_5OH, NaOH

Scheme 6. 1-methyl, 2,3,N-trisubstituted norharmane.

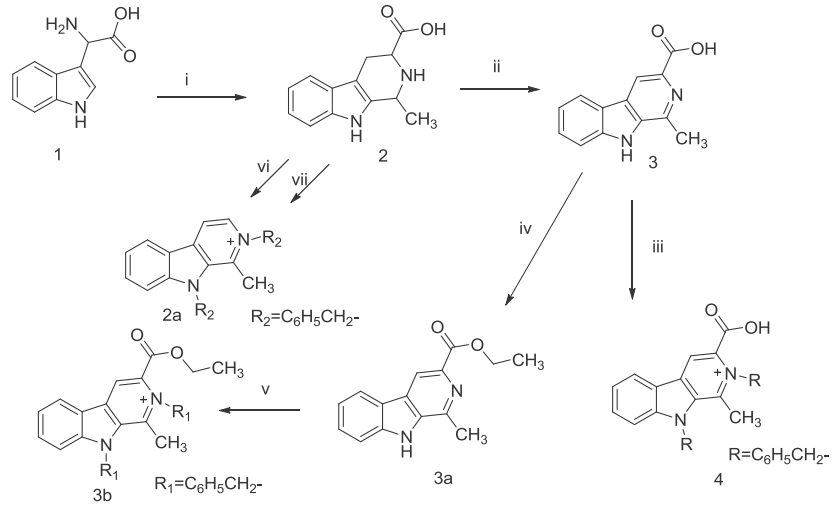
[3,4-*b*]indol-2-ium bromide) was prepared by benzylation of 1-methyl β-carboline 3-ethylcarboxylate 3a in the presence of sodium hydride in anhydrous DMF solution. The product, 4 (2,9-dibenzyl-9H-pyrido[3,4-*b*]indol-2-ium bromide) was synthesized from β-carboline by benzylation at position 2 and 9 as above suitable condition. These compounds had prominent cytotoxic activities against tumor cells IC_{50} values lower than 40 μM from preliminary work [28].

2.8. Synthesis of 1-methyl 3,9-disubstituted norharmane

In Scheme-8, the 1-methyl, *N*-butylated β-carboline 3-carboxylate 5 was synthesized in the reaction with *n*-butyl bromide and sodium hydride in DMF. The obtained precursor 5 was treated with several amines in the presence of DMF to yield 3- (*N*-substituted amino) 1,9 disubt. β-carboline carboxamide in which, the compound 6 was prepared by the mixture of an intermediate ester i.e., the compound methyl 1-benzyl-9-methylβ-carboline 3-carboxylate with ethylenediamine in DMF solution. The compound 6 had DNA intercalating ability along with cytotoxicity to tumor cell lines, which possess S and G2-M arresting effect in Hela cells [29].

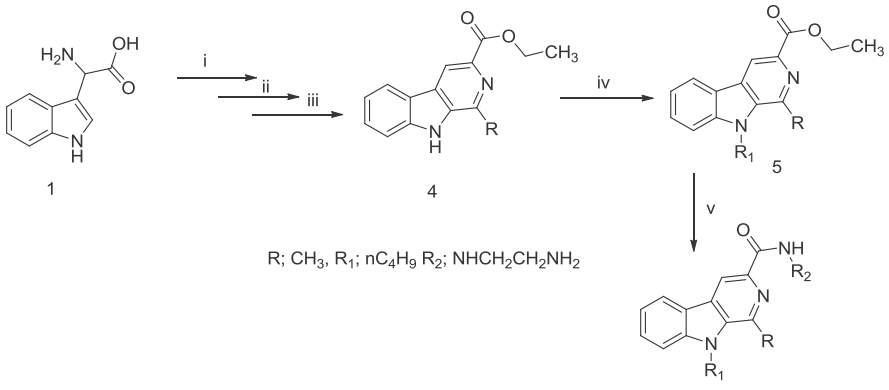
2.9. Synthesis of N-butyl norharmane 3-carboxylic acid

In Scheme-9, an 9-alkyl and benzyl derivative of norharmane was prepared from the starting amino acid tryptophan, by its reaction with formaldehyde and its derivative via the well-known reaction, Pictet-spengler's condensation, which further was treated with *n*-butyl bromide in the presence of sodium hydride in



Reactions: (i) H^+, CH_3CHO; (ii) $K_2Cr_2O_7$, CH_3COOH; (iii) $C_6H_5CH_2Br$, NaH, DMF; (iv) H^+, C_2H_5OH; (v) $C_6H_5CH_2Br$, NaH, DMF; (vi) HAc, SeO_2; (vii) $C_6H_5CH_2Br$, NaH, DMF

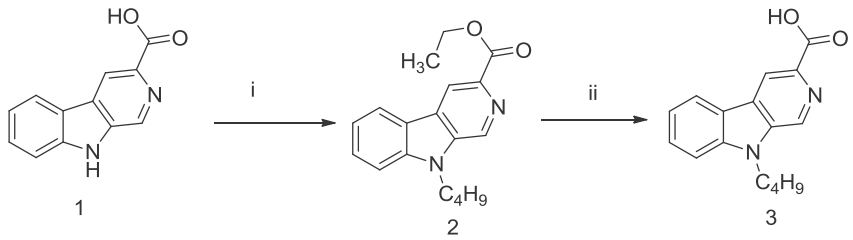
Scheme 7. 1-methyl, 2,3, N-trisubstituted norharmane.



Reactions: (i) H^+,OH^-, RCHO; (ii) H^+,C_2H_5OH ,RT; (iii) S, Xylene, RT; (iv) n C_4H_9Br, NaH, DMF; (v) $NH_2CH_2CH_2NH_2$, C_2H_5OH,DMF

Scheme 8. 1-methyl 3,9-disubstituted norharmane.

DMF solution. Immediately, the product an esterified with alcohol in acid to produce 1, 2, 3, 4-tetrahydro β-carboline 3-carboxylate derivatives, which on oxidative decarboxylation with selenium dioxide in acetic acid yielded β-carboline derivative. Finally, the ester product was hydrolysed in alkali to produce 9-butyl, β-carboline 3-carboxylic acid 3. The compound 3, having an *n*-butyl and a carboxyl group at N-9 and C-3 positions had the highest antitumor effect along with the lowest acute toxicity and neurotoxicity. The tumor inhibition was approximately 40% against mice bearing Lewis lung cancer and another cell line, S180; and it had the highest antitumor effect with the tumor inhibition 46.9% against mice having Lewis lung cancer [30].



Reactions: (i) NaH,C_4H_9I; (ii) NaOH

Scheme 9. N-butyl norharmane 3-carboxylic acid.

2.10. Synthesis of 1-methyl 3-substituted N-propyl, benzyl, hydroxamate norharmane

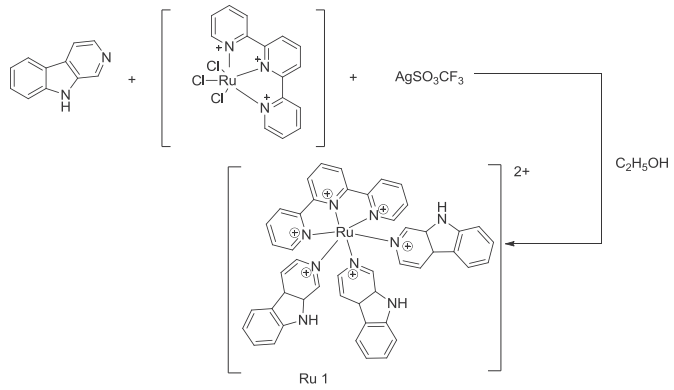
In Scheme-10, a series of β-carboline hydroxamate derivative as a potent histone deacetylase inhibitor had been synthesized and the structure was interpreted. The compound 6 was prepared from an intermediate methyl 4-((1-(4-methoxyphenyl)–*N*-propyl-9H-pyrido[3,4-*b*]indole-3-carboxamido)methyl)benzoate 7 mixed with NH_2OK in methyl alcohol. The intermediate amides products were synthesized by treatment with the compound 1-substituted β-carboline 3- carboxylic acid 4 along with substituted amines in presence of reagent, 1-ethyl -3-(3-dimethylaminopropyl) carbodiimide hydrochloride(EDCI) and 4-dimethylamino-pyridine. Compound 6 had showed the strongest inhibition of the proliferation of human hepatocellular carcinoma (HCC) cells *in vitro*, with IC_{50} values. It induced 61.8% induction of apoptosis in HepG2 cell with 4.0 μM of the compound 6 was significantly 5.0 μM with 34.5% apoptotic cells [31].

2.11. Synthesis of Ruthenium-norharmane metal complex

Similarly, the Scheme 11 is a complex compound Ru1 was prepared by the additional mixtures of $Ru(tpy)Cl_3$ with $AgSO_3CF_3$ in ethanol, which was followed by the addition of norharmane in the reaction mixture. The Ru(II) has 3 norharmane molecules in its 3 bonds and the two remaining bonds were attached to one substitute of norharmane, like a clamp connection, The Ru(II) metal-based complexes developed is a potent anticancer agent, which is efficient than cisplatin. The compound Ru1 had activation of caspase-9 and capsase 3/7; and it induced apoptosis via p53mediated pathway and inhibited MCF-7and HepG2 tumor cells [32].

2.11.1. Structures of Ruthenium norharmane complex

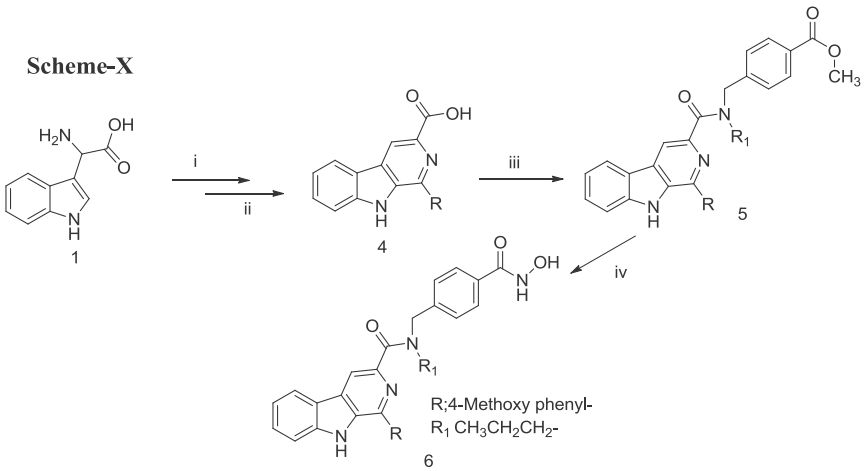
In Scheme 12, the three novel Ruthenium(II) complexes (1–3) were synthesized, whose general structure is Ru-(*N*–N)$_2$(norharman)$_2$ SO_3CF_3, where the addition of *N*–N = 2,2′-bipyridine (bpy), 1,10 phenanthroline (Phen) and 4,7-diphenyl 1,10-phenanthroline (DIP). Moreover, the complexes 1 and 2 bear bipyridyl and phenanthroline, respectively. Notably, all these compounds exhibit potent antiproliferative activities against a number of human carcinoma cell lines. whereas complex 3 was seen with the lower IC_{50} value than that of standard Cisplatin. The lipophilic complex 3, with an ancillary ligand DIP had comparatively a higher efficiency in cellular uptake, and it triggers the arrest of cell cycle at G0/G1. Eventually, the subsequent apoptosis via mitochondrial dysfunction and the accumulation of reactive oxygen species (ROS) [33].



Scheme 11. Ruthenium-norharmane metal complex.

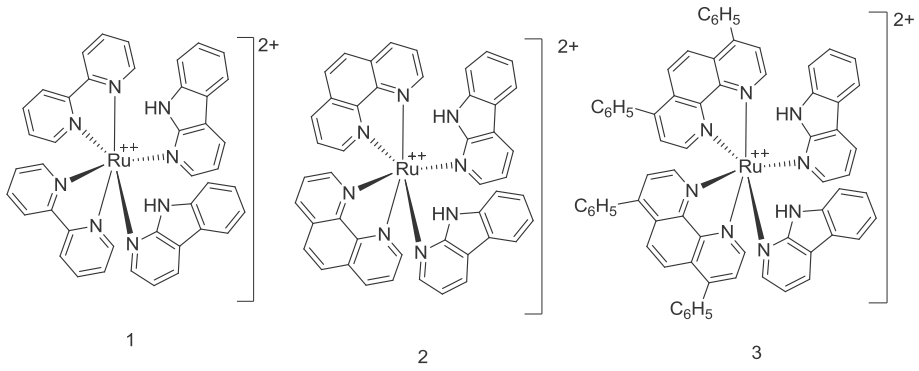
2.11.2. Structures of Ruthenium complex bearing ancillary ligands

In Scheme 13, the cited four Ru (II) Ruthenium-norharmane complexes were prepared from the precursors, whose general form was [Ru(tpy) (*N*–N) Cl] Cl in which, (*N*–N) = bpy, phen, dpa and dip. The complex Ru1 was synthesized by mixing [Ru(tpy) (bpy) Cl] Cl with $AgSO_3CF_3$ in ethanol under reflux for 12 h to which norharmane was further added in the reaction mixture that was refluxed for 12 h for the final desired complex, [Ru(tpy) (bpy) Nh] $(CF_3SO_3)_2$. Similarly, the complex Ru 2 [Ru(tpy) (phen) Nh] $(CF_3SO_3)_2$ was prepared by the mixture of [Ru(tpy) (phen) Cl] Cl and $AgSO_3CF_3$ followed by mixing norharmane with the mixture. Other two complexes Ru3 and Ru4 obtained αom [Ru(tpy) (dpa) Cl] Cl and [Ru(tpy) (dip) Cl] Cl as described here above. Those complexes were induced to activate apoptosis of human cancer cells, against caspase-9 and caspase-3/-7. Likewise, all those complexes exhibited a broad spectrum of inhibition on cancer cells, with IC_{50} values ranging from 15 to 60 μM [34].

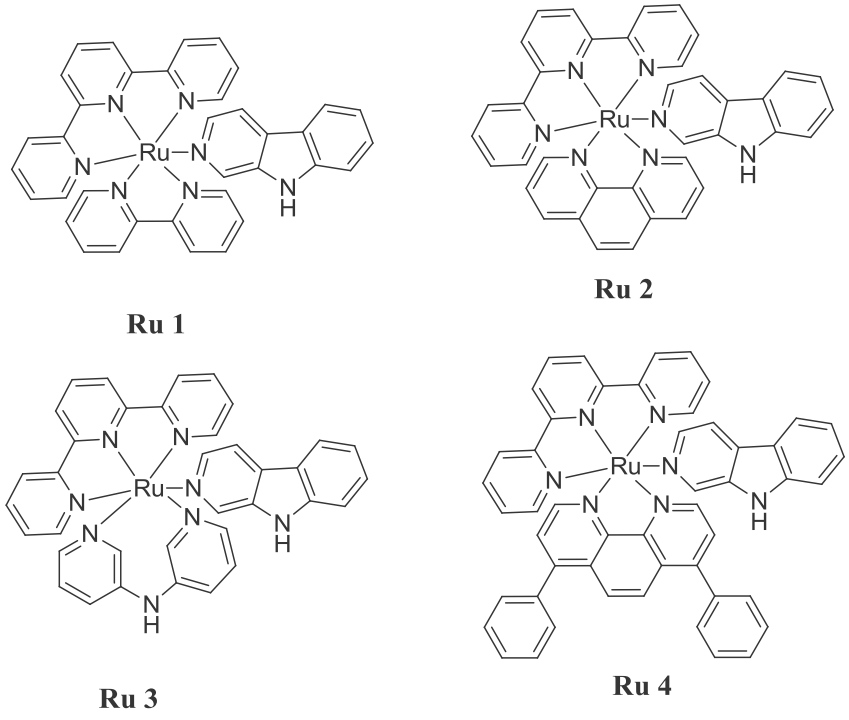


Reaction: (i) H^+,OH^-, RCHO, reflux, 2-4h; (ii) $KMnO_4$,DMF,RT, 6h; (iii) EDCl,DMAP, CH_2Cl_2, Rt, 5-8h; (iv) NH_2OK, CH_3OH, Rt, 10-15h

Scheme 10. 1-methyl 3-substituted N-propyl, benzyl, hydroxamate norharmane.



Scheme 12. Ruthenium norharmane complex.



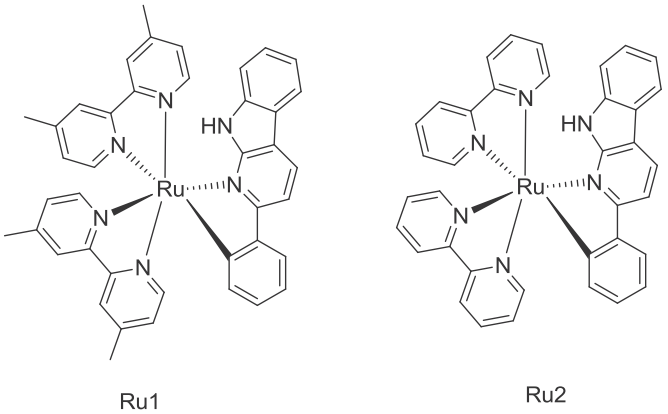
Scheme 13. Ruthenium complex bearing ancillary ligands.

2.11.3. *Structures of Ruthenium norharmane complex contains dmb and bpy*

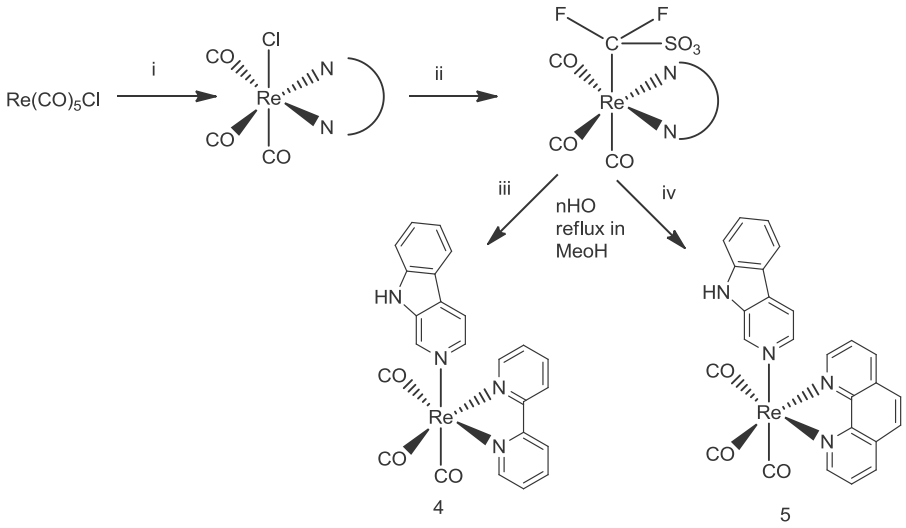
In Scheme 14, both Ru1 and Ru2 complexes were synthesized individually by the reaction of cis-Ru(*N*–N)$_2$Cl$_2$ (*N*–N = dmb, bpy) with 1-phenyl β-carboline in ethanol at room temperature; the desired complexes were isolated and purified. Ru1 was more potent than Ru2 related to action against human cancer cell lines. The coordination of the cyclometalated ligand 1-phenyl β-carboline to polypyridyl-Ru centres was seen comparatively more significant in cytotoxicity [35].

2.12. *Synthesis of Rhenium-norharmane metal complex*

In Scheme-15, both complexes of Re(CO)$_3$bpy CF_3SO_3 and Re(CO)$_3$ phen CF_3SO_3 were intermediate precursors from which, the final two products Rhenium-norharman metal complex. [Re(CO)$_3$(bpy) (nHo)] CF_3SO_3 and [Re(CO)$_3$(phen) (nHo)] CF_3SO_3, were obtained by treating individually with norharmane under reflux in toluene in nitrogen atmosphere [36].



Scheme 14. Ruthenium norharmane complex contains dmb and bpy.



Reaction : (i) bpy or phen, Reflux in toluene; (ii) $AgCF_3SO_3$, Reflux in THF; (iii) bpy, Reflux in MeOH; (iv) phen, Reflux in MeOH

Scheme 15. Rhenium-norharmane metal complex.

2.12.1. Structures of Rhenium(I) tricarbonyl norharmane complex

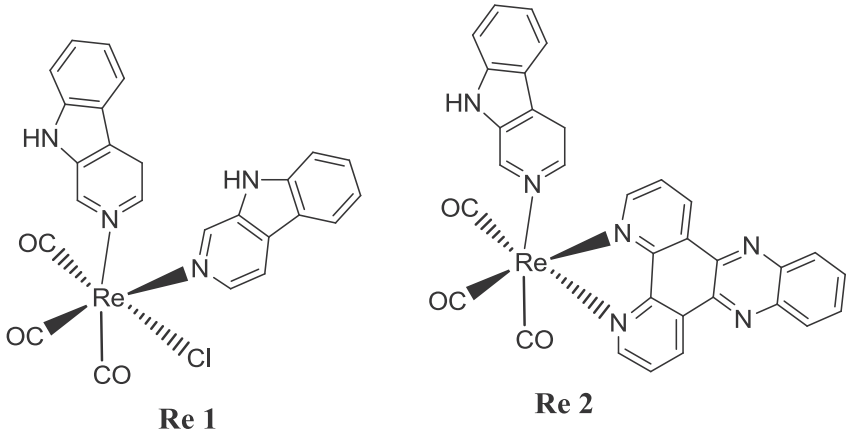
In Scheme 16, the synthesis of the compound Re1, [Re $(CO)_3(nHo)_2$] Cl was obtained by the mixing Re $(CO)_5Cl$ and norharman(nHo) with the ratio 2:1, and the mixture was heated under reflux in toluene in presence of nitrogen atmosphere for 8 h. The desired complex was recrystallized from dichloromethane (DCM). Furthermore, the compound Re2, [Re $(CO)_3$(dppz) (nHo)] SO_3CF_3 was synthesized as earlier reported and it had the highest cellular uptake and cytotoxicity activity. Both complexes were reported effective against human lung carcinoma (A549), which in comparison to the standard cisplatin with a similar IC_{50} value [37].

2.13. Synthesis of spiropiperdine ring possessing reduced norharmane nucleus

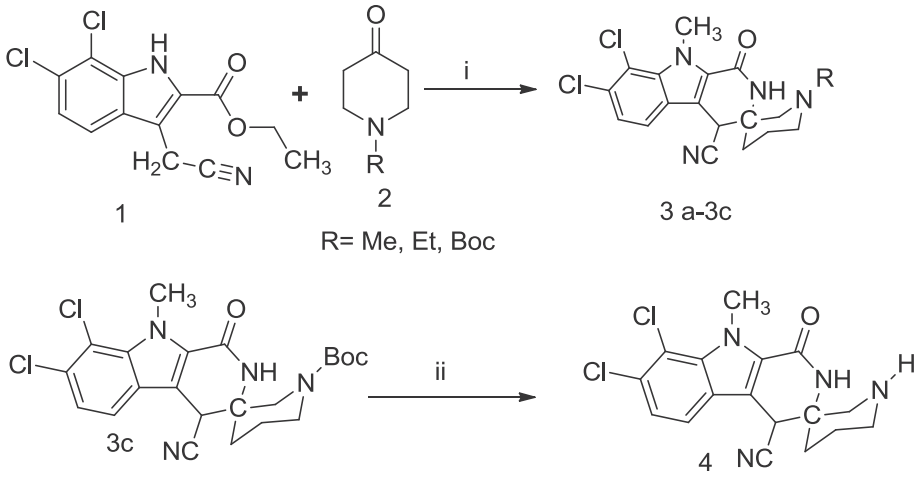
In Scheme 17, a novel chemical class of substituted 7,8-dichloro,1-oxo- β-carboline were synthesized, basing on the structural alkaloid bauerine. Among all the congeners, the compound 3 was found to PIM-dependent phosphorylation of downstream effector proteins and had a comparatively a better antiproliferative action, which may be due to the formation of scaffold with 6,7-dichloroindole nucleus. The compound 4, (3S)-7′,8' -dichloro-9′methyl-1′-oxo-1′,2′,4′,9′-tetrahydrospiro[piperidine-3,3′-pyrido[3,4-b] indole]-4′-carbonitrile was synthesized from the intermediate, ethyl 3-cyanomethyl-1H-indole-2-carboxylate 1 with Boc-protected piperidone 2c in gaseous ammonia or ammonium chloride that further was deprotected by using trifluoroacetic acid in DCM [38].

2.14. Other norharmane derivatives

The substituted β-carbolinium bromide compounds were prepared and among all products the compound 1 9-benzyl-2-butyl-1-(2-chlorophenyl)- β-carbolineum bromide and compound, compound 2 (DH_{335}) 9-benzyl-2-butyl-β-carbolinium bromide had a better potency on enzyme inhibition [39]. Another phytochemical an alkaloid, evodimine, isolated from the Chinese herb, *Evodia rutaecarpa,* was fused with norharmane moiety. The fusion product was reported to possess several biological actions including the anticancer potentiality, viz., inhibiting the angiogenesis and



Scheme 16. Rhenium(I) Tricarbonyl norharmane complex.



Scheme 17. Spiropiperdine ring possessing reduced norharmane nucleus.

metastasis in tumor cell lines, in addition to stimulation of autophagy for the survival function [40]. The ruthenium Ru (II) complexes whose general formula [Ru(*N*–N) (norharmane)$_2$](SO_3CF_3)$_2$, where *N*–N = 2,2-bipyridine(bpy,1),1,10 phenanthroline (phen,2), 4,7-diphenyl-1,10-phenanthroline(DIP,3) (Fig. 2). These complexes were obtained by refluxing with appropriate precursor and norharmane in aqueous methanol [41].

The photodynamic treatment (PDT) is utilized in assessing cytotoxicity of chemicals in tumor cells. Two novel β-carbolinium subsidiaries, 2-(2-carboxyethyl)- norharmanium (cnHo) and 2-(2-carboxyethyl)- harminium (cHa) cations were the chosen photosensitizers to explore the activity folate receptor α (FRα) for phototoxicity in KB carcinoma cell lines. Furthermore, the cHa was used for assessing cytotoxicity, while the other subsidiary was utilized as chromophore for the fluorescence based test segment. The mixtures of subsidiaries in quaternary structures turned polar, limiting the free photosensitization. Experimentally, those were covalently connected to albumin with carbodiimide and those products were coupled, eventually to separately deliver cnHo-albumin and nHa-albumin. In the next step, cnHo-albumin-FA and nHo-albumin-FA separately were generated from conjugates being coupled to folic acid (FA). Phototoxic and nontoxic results were reported using 'folate-albumin complex with β-carbolinium' and without the complex, respectively [42].

3. SAR of norharmane derivatives with anticancer activity

In the presence of functionality in norharmane nucleus is a main component, which substituents or as side-chain alteration in several positions, has encouraged the synthesis chemists to develop new anticancer compounds bearing this moiety. SAR studies on derivatives of norharmane compounds and their anticancer activity discussed and illustrated (Fig. 3).

The 9-substituted norharmane molecules possess maximum activity against a sirtuin5, which as a class of exhibit enzymes, deacetylases, which perform the deacetylation by using nicotinamide adenine dinucleotide as a cofactor in regulating mitochondrion metabolism is reported as a critical factor against cancer target [22]. Moreover, 9-substituted, enhancing the DNA intercalating ability and inhibition of DNA topoisomerase and cyclin dependent kinase of cancer HeLa and BGC-823 cell lines [29].

Most of transitional metal complexes exhibit a wide range of

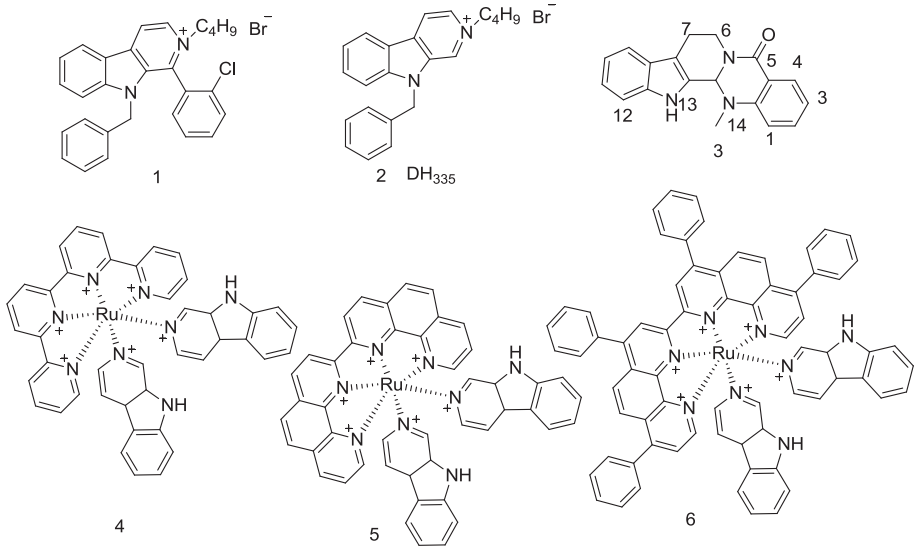


Fig. 2. Anti-cancer activity of lead molecule from several prescribed schemes.

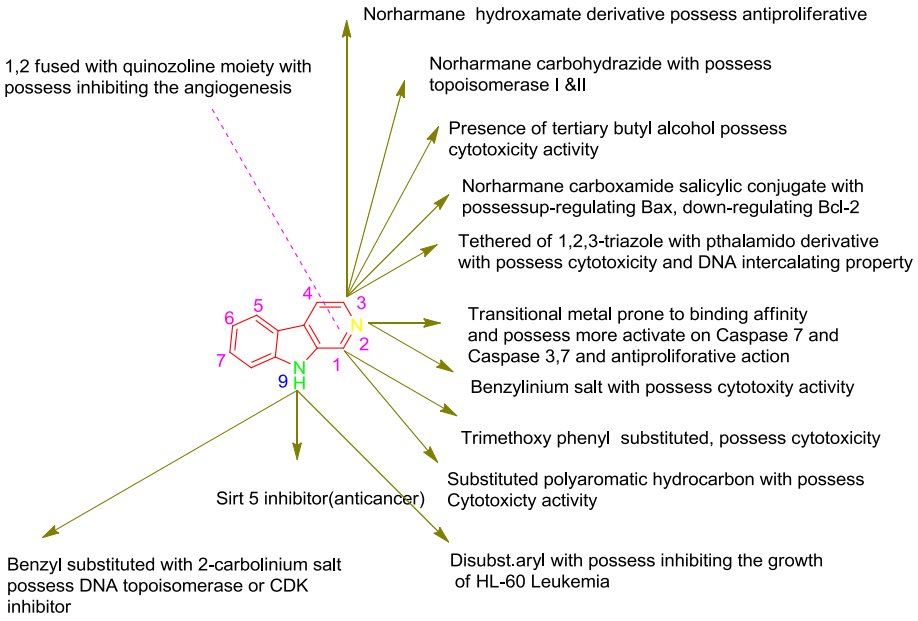


Fig. 3. SAR of norharmane and its derivative against cancer.

biological applications, but those are less developed for cellular uptake into any carcinoma cells for example, HeLa cells, except Ruthenium metal complexes. Ru complexes have ancillary ligands with a greater lipophilicity and a significant cellular uptake efficacy with comparison to cisplatin [43].

Ruthenium-II (Ru)-norharmane complex and their derivatives such as, tris chelate polypyridyl [41] possess significant activation on caspase-9 and caspase 3/7, which induce apoptosis via p53mediated pathway. The antiproliferative action having a lower IC_{50} value than the stranded Platinum based complex, cisplatinum [32]. The C-3 position several molecules of the β-carboline moiety, linked with 1, 2, 3-triazole nucleus is exhibited promising cytotoxicity and DNA intercalating property [24]. The presence of a phthalimide or naphthalimide pharmacophore linked through a 1,2,3-triazole at C3-position might played a key role in the modulation of cytotoxic potencies.

Likewise, Ruthenium metal complexes contain either polypyridyl or norharmane moiety as an ancillary ligand. Those complexes exhibit cellular uptake efficiency and cytotoxicity which were reported to increase lipophilic character, and the aromatic surface area of the ligand (*N*–N) were with increasing trends [44].

The norharmane-salicylic conjugate amides had significant control of liver and colon cancer cells *in vitro*, which were recorded as more effective than 5-fluro uracil (pyrimidine antagonist); norharmane-salicylic conjugate amides are associated with mitochondrial depolarization in liver cancer cells by up regulating Bax [27]. The hybrid link between butane diamine and amyl diamine might have exhibited a better antitumor activity than that by propane diamine and/or hexanediamine. Furthermore, 2, 6-disubstituted norharmane congeners are also potent cancer enzyme inhibitors. Polyaromatic groups (naphyl and phenanthryl) attached at C-1 position of norharmane synthesized conjugates also resulted in remarkable cytotoxicity against five cancer cell lines [26].

Introduction of substituent 2-methoxy benzyl at N-9 position and attached to carboxylic group at C-3 in norharmane lead product had remarkable anticancer activity against HL-60 [27]. Introducing the appropriate substituents into position-9 and 2 of norharmane derivatives increased greatly antitumor activities and at position-3 and 1 significantly contributed to the decreased acute toxicity of norharmane derivatives; the structural framework was an important derived compound for design and synthesis of novel antitumor agents. β-carboline hydroxamate derivatives were potent histone deacetylase inhibitors and had antiproliferative activity [31].

The review has explained herewith a lot of synthetic strategies and anticancer activities, against specific enzyme and it elaborates the SAR of norharmane moiety.

4. Present and future prospective of natural product derivatives for anticancer drug development

Today, employing natural secondary metabolites from several sources including marine resources, locating the suitable chemical against the gargantuan types of human cancer is a herculean task. But recent medicinal chemistry approaches offer ancillary techniques for designing chemicals of curio for therapeutic benefits in respect to cancer treatment. Moreover, phyto-extracts of ashwagandha, brassica along with ginseng, curcumin, genistein, lutein, quercetin, epigallocatechin gallate and sulforaphane are in the clinical trials as anticancer natural remedy. Similarly, the synthetic strategies of norharmane and its derivatives could be designed for newer anticancer drugs. As this is an emerging area of research, further work is required to fully understand the plausible uses norharmane and its derivative, which could be developed a novel anticancer drug for pharmaceutical sector. Blithely, norharmane and structurally alternated derivatives control cancer. The described norharmane derivatives with altered positions were at active sites, C-1, C-3, and N-9, while other sites could be further be explored for developing a potent drug candidate(s) against cancer cell lines.

In addition to, indole-3-carbinol and its derivative diindolylmethane from *Brassica* vegetables, broccoli, cauliflower and collard greens were reported as effective anticancer agents against, breast, prostate and ovarian [45]. Currently both indole derivatives are under clinical trials particularly, di-indolylmethane targets cycle arrest, altering angiogenesis, invasion, metastasis and epigenetic behavior of cancer cells trough NF-κB/Wnt/vAkt/mTOR pathways

[46], whereas, indole-3-carbinol targets GSTm2, UGT1A1, and NQO1 [47].

However, several chemicals are in pharmacological pipeline at preclinical stage waiting for validation. Norharmane, present in of both plant and marine sources, is a unique chemical entity that would be coming up as drug in the crusade against cancer. Indeed, medicinal chemistry exports design/synthesize robust analogues based on its SARs for enhancing chemo preventive potency. Druggability issue such as, bioavailability, host toxicity patterns and several other favorable characteristics shall be taken up before its promotion to preclinical stage. For example, curcumin and its synthetic analogue-EF24 were validated and around ~10-fold greater potency of the later than the natural form was seen [48]. Herein, the recorded finding on SAR of norharmane and derivatives might be helpful for the further anticancer drug development, when three useful derivatives pass through pharmacological tests.

5. Conclusion

This review explores recent advances of synthesis and the structural activities of lead molecules were discussed. The potent anticancer activities of norharmane derivative against several cell lines are summarized. Natural and synthesized norharmane derivatives have potent and those could be regarded as lead candidates for anticancer based on SAR. Moreover, the conventional synthetic method included Pictet-splengler and Bischler-napieralsky reactions, which had been considered as the primer syntheses of precursor norharmane nucleus.

Conflicts of interest

The authors declare that they do not have any conflict of interest.

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References

[1] Anonyms, Key Facts on Cancer, WHO, 2018. http://www.who.int/news-room/fact-sheets/detail/cancer. (Accessed 30 July 2018).
[2] S. Rajpal, A. Kumar, W. Joe, Economic burden of cancer in India: evidence from cross-sectional nationally representative household survey, 2014, PLoS One 13 (2) (2018) 1–17.
[3] M. Fridlender, Y. Kapulnik, H. Koltai, Plant derived substances with anticancer activity: from folklore to practice, Front. Plant Sci. 6 (2015) 799.
[4] H. Yuan, Q. Ma, L. Ye, G. Piao, The traditional medicine and modern medicine from natural products, Molecules 21 (2016) 559.
[5] D. Dubey, S. Rath, M.C. Sahu, N.K. Debata, R.N. Padhy, Antimicrobials of plant origin against TB and other infections and Economics of plant drugs –Introspection, Ind. J. Tradit. Knowl. 11 (2012) 225–233.
[6] S.S. Swain, S.K. Paidesetty, R. Padhy, Computational attempts for synthesis of potent antibacterial sulfamethoxazole-monocyclic terpenes conjugates, Can. J. Biotech. 1 (2017), 52-52.
[7] S.S. Swain, R.N. Padhy, P.K. Singh, Anticancer compounds from cyanobacterium *Lyngbya* species- a review, Antonie Leeuwenhoek 108 (2015) 223–265.
[8] T. Karan, R. Erenler, Screening of norharmane from seven cyanobacteria by high-performance liquid chromatography, Phcog. Mag. 13 (2017) 723–725.
[9] J.R.F. Allen, B.R. Holmstedt, The simple β -carboline alkaloids, Phytochemistry 19 (1980) 1573–1582.
[10] F.A.O. Rasse-Suriani, F.S. García-Einschlag, M. Rafti, T.S. De-León, P.M.D. Gara, R. Erra-Balsells, F. M, Cabrerizo Photophysical and photochemical properties of naturally occurring normelinonine F and Melinonine F alkaloids and structurally related N(2)- and/or N(9)-methyl-β-carboline derivatives, Photochem. Photobiol. 94 (2018) 36–51.
[11] R.B. Volk, Screening of microalgae for species excreting norharmane, a manifold biologically active indole alkaloid, Microsc. Res. 163 (2008) 307–313.
[12] M.S. Siderhurst, D.M. James, C.D. Rithner, D.L. Dick, L.B. Bjostad, Isolation and characterization of norharmane from *Reticulitermes termites* (Isoptera: Rhinotermitidae), J. Econ. Entomol. 98 (2005) 1669–1678.
[13] Z.A. Mohamed, Allelopathic activity of the norharmane-producing cyanobacterium *Synechocystis aquatilis* against cyanobacteria and microalgae, Oceanol. Hydrobiol. Stud. 42 (2013) 1–7.
[14] R.B. Volk, Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*, J. Appl. Phycol. 17 (2005) 339–347.
[15] T. Karan, R. Erenler, Screening of norharmane from seven cyanobacteria by high-performance liquid chromatography, Phcog. Mag. 13 (2017) 723–725.
[16] K. Yomosa, A. Hirota, H. Sakai, A. Isogai, Isolation of harman and norharman from *Nocardia* sp. and their inhibitory activity against plant seedlings, Agric. Biol. Chem. 51 (1987) 921–922.
[17] C.A. Bourke, G.R. Stevens, M.J. Carrigan, Locomotor effects in sheep of alkaloids identified in Australian *Tribulus terrestris*, Aust. Vet. J. 69 (1992) 163–165.
[18] J.Q. Leclercb, L. Angenot, N.G. Bisset, South American *Strychnos* species. ethnobotany (except curare) and alkaloid screening, J. Ethnonopharm. 28 (1990) 1–52.
[19] H. Tsuchiya, H. Shimizu, M. Iinuma, β-carboline alkaloids in crude drugs chem, Pharm. Bull. 47 (1999) 440–443.
[20] T. Herraiz, Identification and occurrence of the bioactive β-carbolines norharman and harman in coffee brews, Food Addit. Contam. 19 (2002) 748–754.
[21] H. Tomas, C. Carolina, Human monoamine oxidase enzyme inhibition by coffee and β-carbolines norharman and harman isolated from coffee, Life Sci. 78 (2006) 795–802.
[22] L. Yang, Y. He, Q.L. Chen, S. Qian, Z.Y. Wang, Design and synthesis of new 9-substituted norharmane derivatives as potential Sirt5 inhibitors, J. Heterocycl. Chem. 54 (2016) 1457–1466.
[23] S. Nagula, R. Tokala, S. Thatikonda, S. Sana, U.S. Vanteddu, C. Godugu, Design and synthesis of DNA-interactive β-carboline-oxindole hybrids as cytotoxic and apoptosis inducing agents, ChemMedChem 19 (2018) 1909–1922.
[24] N. Shankaraiah, C. Jadala, S. Nekkanti, K.R. Senwar, N. Nagesh, S. Shrivastava, V.G.M. Naidu, M. Sathish, A. Kamal, Design and synthesis of C3-tethered 1,2,3-triazolo-β-carboline derivatives: anticancer activity, DNA-binding ability, viscosity and molecular modeling studies, Bioorg. Chem. 64 (2016) 42–50.
[25] Q.B. Xu, X.F. Chen, J. Feng, J.F. Miao, J. Liu, F.T. Liu, B.X. Niu, J.Y. Cai, C. Huang, Y. Zhang, Y. Ling, Design, synthesis and biological evaluation of hybrids of β-carboline and salicylic acid as potential anticancer and apoptosis inducing agents, Sci. Rep. (2016) 1–12.
[26] B. Bai, X.Y. Li, Y. Li, H.J. Zhu, Syntheses of novel β-carboline derivatives and the activities against five tumor-cell lines, Bioorg. Med. Chem. Lett. 24 (2014) 96–98.
[27] Y.F. Chen, Y.C. Lin, J.P. Chen, H.C. Chan, M.H. Hsu, H.Y. Lin, S.C. Kuo, L.J. Huang, Synthesis and biological evaluation of novel 3,9-substituted β-carboline derivatives as anticancer, Bioorg. Med. Chem. Lett. 25 (18) (2015) 3873–3877.
[28] R. Cao, H. Chen, W. Peng, Y. Ma, X. Hou, H. Guan, X. Liu, A. Xu, Design, synthesis and *in vitro* and *in vivo* antitumor activities of novel β-carboline derivatives, Eur. J. Med. Chem. 40 (2005) 991–1001.
[29] H. Guan, H. Chen, W. Peng, Y. Ma, R. Cao, X. Liu, A. Xu, Design of β-carboline derivatives as DNA-targeting antitumor agents, Eur. J. Med. Chem. 41 (2006) 1167–1179.
[30] R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A. Xu, Synthesis, acute toxicities, and antitumor effects of novel 9-substituted β -carboline derivatives, Bioorg. Med. Chem. 12 (2004) 4613–4623.
[31] J. Liu, T. Wang, X. Wang, L. Luo, J. Guo, Y. Peng, Q. Xu, J. Miao, Y. Zhang, Y. Ling, Development of novel β-carboline-based hydroxamate derivatives as HDAC inhibitors with DNA damage and apoptosis inducing ability, Med. Chem. Commun. 8 (2017) 1213–1219.
[32] Y. Chen, M.Y. Qin, L. Wang, H. Chao, L.N. Ji, A.L. Xu, A Ruthenium(II) β-carboline complex induced p53-mediated apoptosis in cancer cells, Biochimie 95 (2013) 2050–2059.
[33] C. Tan, S. Wu, S. Lai, M. Wang, Y. Chen, L. Zhou, Y. Zhu, W. Lian, W. Peng, L. Ji, A. Xu Synthesis, structures, cellular uptake and apoptosis-inducing properties of highly cytotoxic ruthenium-Norharman complexes, Dalton Trans. 40 (2011) 8611–8621.
[34] Y. Chen, M.Y. Qin, J.H. Wu, L. Wang, H. Chao, L.N. Ji, A.L. Xu, Synthesis, characterization, and anticancer activity of ruthenium(II)-β-carboline complex, Eur. J. Med. Chem. 70 (2013) 120–129.
[35] J. Chen, F. Peng, Y. Zhang, B. Li, J. She, X. Jie, Z. Zou, M. Chen, L. Chen, Synthesis, characterization, cellular uptake and apoptosis-inducing properties of two highly cytotoxic cyclometalated ruthenium(II) β-carboline complexes, Eur. J. Med. Chem. 140 (2017) 104–117.
[36] I. Maisuls, E. Wolcan, O.E. Piro, G.A. Echeverría, G. Petroselli, R. Erra-Balsells, F.M. Cabrerizo, G.T. Ruiz, Norharmane Rhenium(I) polypyridyl complexes: synthesis, structural and spectroscopic characterization, Dalton Trans. 44 (2015) 17064–17074.
[37] I. Maisuls, E. Wolcan, O.E. Piro, E.E. Castellano, G. Petroselli, R. Erra-Balsells, F.M. Cabrerizo, G.T. Ruiz, Synthesis, structural characterization and biological evaluation of Rhenium(I) Tricarbonyl complexes with β-carboline ligands,

Chemistry Select 2 (2017) 8666–8672.
[38] K. Huber, L. Brault, O. Fedorov, C. Gasser, P. Filippakopoulos, A.N. Bullock, D. Fabbro, J. Trappe, J. Schwaller, S. Knapp, F. Bracher, 7,8-Dichloro-1-oxo-β-carbolines as a versatile scaffold for the development of potent and selective kinase inhibitors with unusual binding modes, J. Med. Chem. 55 (2012) 403–413.
[39] Y. Li, F. Liang, W. Jiang, F. Yu, R. Cao, Q. Ma, X. Dai, J. Jiang, Y. Wang, S. Si, DH334, a β-carboline anti-cancer drug, Inhibits the CDK activity of budding yeast, Cancer Biol. Ther. 6 (2007) 1193–1199.
[40] J.J. Lu, J.L. Bao, X.P. Chen, M. Huang, Y.T. Wang, Alkaloids Isolated from natural herbs as the anticancer agents, Evid. Based Comp. Alternat. Med. 2012 (2012) 1–12.
[41] C. Tan, S. Wu, S. Lai, M. Wang, Y. Chen, L. Zhou, Y. Zhu, W. Lian, W. Peng, L. Jib, A. Xu, Synthesis, structures, cellular uptake and apoptosis-inducing properties of highly cytotoxic ruthenium-norharman complexes, Dalton Trans. 40 (2011) 8611.
[42] K. Butzbach, F.A.O. Rasse-Suriani, M.M. Gonzalez, F.M. Cabrerizo, B. Epe, Albumin-folate-conjugates for drug-targeting in photodynamic therapy, Photochem. Photobiol. 92 (2016) 611–619.
[43] C.A. Puckett, J.K. Barton, Methods to explore cellular uptake of ruthenium complexes, J. Am. Chem. Soc. 129 (2007) 46–47.
[44] U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia, S. Wolfl, Cellular uptake, cytotoxicity, and metabolic profiling of human cancer cells treated with ruthenium(II) polypyridyl complexes [$Ru(bpy)_2$ (N-N)] Cl_2 with N-N=bpy, phen, dpq, dppz, and dppn, ChemMedChem 3 (2008) 1104–1109.
[45] C.A. Thomson, E. Ho, M.B. Strom, Chemopreventive properties of 3,3′-diindolylmethane in breast cancer: evidence from experimental and human studies, Nutr. Rev. 74 (2016) 432–443.
[46] S.C. Gupta, J.H. Kim, S. Prasad, B.B. Aggarwal, Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals, Canc. Metastasis Rev. 29 (2010) 405–434.
[47] C.L.L. Saw, M. Cintron, T.Y. Wu, Y. Guo, Y. Huang, W.S. Jeong, A.N.T. Kong, Pharmacodynamics of dietary phytochemical indoles I3C and DIM: induction of Nrf2-mediated Phase II drug metabolizing and antioxidant genes and synergism with isothiocyanates, Biopharm. Drug Dispos. 32 (2011) 289–300.
[48] N. Skoupa, P. Dolezel, E. Ruzickova, P. Mlejnek, Apoptosis induced by the curcumin analogue EF-24 is neither mediated by oxidative stress-related mechanisms nor affected by expression of main drug transporters ABCB1 and ABCG2 in Human Leukemia Cells, Int. J. Mol. Sci. 18 (2017) 2289.