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Synthesis and antibacterial screening of new N-Substituted-9H-β-carboline-6-amine derivatives

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ABSTRACT

β-Carboline is also known as nor-harmane. It is a nitrogen-containing heterocyclic compound formed in plants and animals as Maillard reaction products between amino acids and reducing sugars or aldehydes. These tricyclic nitrogen heterocyclics play a vital role in medicinal chemistry, due to significant biological activities of their derivatives. It is also a key pharmacophore present in a large number of natural tricyclic alkaloids. Current work is reported with the synthesis and antibacterial activity screening of a new series of N-Substituted-9H-β-carboline-6-amine derivatives. The title compounds were synthesized according to the well known Pictet Spengler reaction in three steps by taking 5-Chlorotryptamine and glyoxalic acid as starting materials. This is an acid-catalyzed intramolecular condensation of an iminium ion and an aromatic C-nucleophile which resulted in the formation of 6-Chloro-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indol-2-ium-1-carboxylate (3). Oxidative decarboxylation and aromatization of compound 3 with iodobenzene diacetate led to the 6-Chloro-β–carboline (4) which were treated with different mono substituted amines gave the title compounds (5 a-J). Structures of the synthesized entities were confirmed spectroscopically (FT-IR, ^{1}H NMR and Mass) and screened for antibacterial activity against various pathogenic bacterial strains (Streptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa) by disc diffusion method. The title compounds showed moderate to good antibacterial activity.

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INTRODUCTION

β-Carbolines are a class of synthetic and naturally occurring 2,3-Benzopyrrole alkaloids, consist of azine ring that is fused to 2,3-Benzopyrrole. (Lin *et al.*, 2010; Ratsch, 2005) and endowed with a broad spectrum of essential biochemical and pharmacological functions (Cao *et al.*, 2007; Bernardo *et al.*, 2012) includes anti-platelet aggregation, inhibition of platelet activation (Yao *et al.*, 2011; Liu *et al.*, 2010), acute ischemia (Bi *et al.*, 2011), trypanocidal activity (Costa *et al.*, 2011; Valdez *et al.*, 2012) antiparkinson (Polanski *et al.*, 2011), anti-

cancer (Dighe *et al.*, 2015), antioxidant (Pari *et al.*, 2000) etc.

Pictet Spengler reaction is one of the most obvious ways for construction of tricyclic β–Carboline heterocyclic framework. (Cox and Cook, 1995). The different biological potential of β–Carbolines and the importance of the search for new antibacterial agents have led us to study this class of compounds.

Current work is reported with the synthesis of a title compounds from the well known Pictet Spengler reaction by taking 5-Chlorotryptamine and glyoxalic acid as starting materials, characterization by spectroscopically (FT-IR, ^{1}H NMR and Mass) and screened for antibacterial activity (Savariz *et al.*, 2010).

Experimental

Melting points were determined on a capillary melting point apparatus and are uncorrected. The progress of the reactions was monitored by thin-layer chromatography on silica gel plates. Column chromatography was performed on silica gel. IR spectra were measured in KBr on Bruker spectrophotometer.

^{1}H-NMR spectra were recorded in a Bruker spectrometer at 400MHz (DMSO). Mass spectra were recorded in an APCI Mass spectrometer (APCI-AtmosphericPressure Chemical Ionization). All reagents were purchased from commercial suppliers.

General procedures

Scheme 1 shows that

1. 5-Chlorotryptamine;
2. Oxoacetic acid monohydrate; 3. 6-Chloro-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-b]indol-2-ium-1-carboxylate; 4. 6-Chloro- β-carboline; 5a-j. N-Substituted-9*H*-pyrido[3,4-b]indol-6-amines.

R: 5a. -CH_3; 5b. –CH_2CH_3; 5c. –$CH_2CH_2CH_3$; 5d.-$CH(CH_3)_2$; 5e. –CH_2 $(CH_2)_2CH_3$; 5f. –$CH_2CH(CH3)_2$; 5g. –C_6H_5; 5h. –C_6H_4 (4-NO_2); 5i. –C_6H_4 (4-CH_3); 5j. – Furfuryl.

Synthesis of 6-Chloro-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indol-2-ium-1-carboxylate (3)

In a 1 liter Erlenmeyer flask, 5-Chlorotryptamine 2.31g (0.01 mol) was taken and dissolved in 400ml water by stirring (boiling up to 45^{o}C) and cooled to room temperature. Glyoxalic acid monohydrate (oxoacetic acid) 0.9ml (0.01 mol) was dissolved in 30ml of water.

The glyoxalic acid was added to 5-Chlorotryptamine hydrochloride and the pH of the mixture was adjusted to 3.5-4.0 by slowly adding solution of potassium hydroxide. Cake was formed after stirring at room temperature for 2h, then washed with water and dried.

Yield: 52.6%, Melting point 260^{o}C, Rf Value 0.72, Mol formula $C_{12}H_{11}N_2ClO_2$, Mol Weight 250.68. ^{1}H NMR (400MHz, DMSO)**:** δ 0.99 (2H, t), 1.40 -1.49 (3H, m), 3.61 (1H, s), 7.37-7.41 (1H, m), 7.61 (1H, s), 8.25 (1H, d), 9.57 (1H, s), 10.25 (1H, s). Mass m/z **:** 251 (M+1).

Synthesis of 6-Chloro-9H-β-carboline (4)

Compound 3 (1mmol) was taken in a beaker and dissolved in dimethyl formamide (DMF). To this mixture Iodobenzene diacetate (2 mmol) was added. Which was stirred for 1h at RT.

Completion of reaction was monitored by TLC. The resulting mixture was quenched by saturated $NaHCO_3$ solution and extracted with $CH_3COOC_2H_5$.

All organic layers were combined and washed with brine and dried over $MgSO_4$ and filtered. Solvent was evaporated by rota evaporator.

Obtained residue was purified by column chromatography using ethyl acetate and hexane as solvent system in increasing order of their polarities.

Yield: 76.5%, Melting point 280^{o}C, Rf Value 0.54, Mol formula $C_{11}H_7N_2Cl$, Mol Weight 202.63. ^{1}H NMR (400MHz, DMSO): δ 7.36-7.40 (1H, m), 7.49 (1H, s), 7.54-7.58 (2H, m), 8.06 (1H, d), 8.50 (1H, s), 10.32 (1H,br s, NH). Mass m/z: 203 (M+1).

Synthesis of N-Substituted-9H-β-carbolin-6-amine derivatives (5a-j)

N-Substituted amine (1mol) was added to a solution of 6-Chloro-9H-β-carboline (1mol) in anhydrous toluene (5.0ml). The above mixture was stirred at RT for 20-24h.

Under reduced pressure, excess toluene was distilled off from the reaction mixture.

The mixture was poured into crushed ice and further neutralized with HCl.

The resultant precipitate was filtered; air dried and recrystallized using ethanol as solvent.

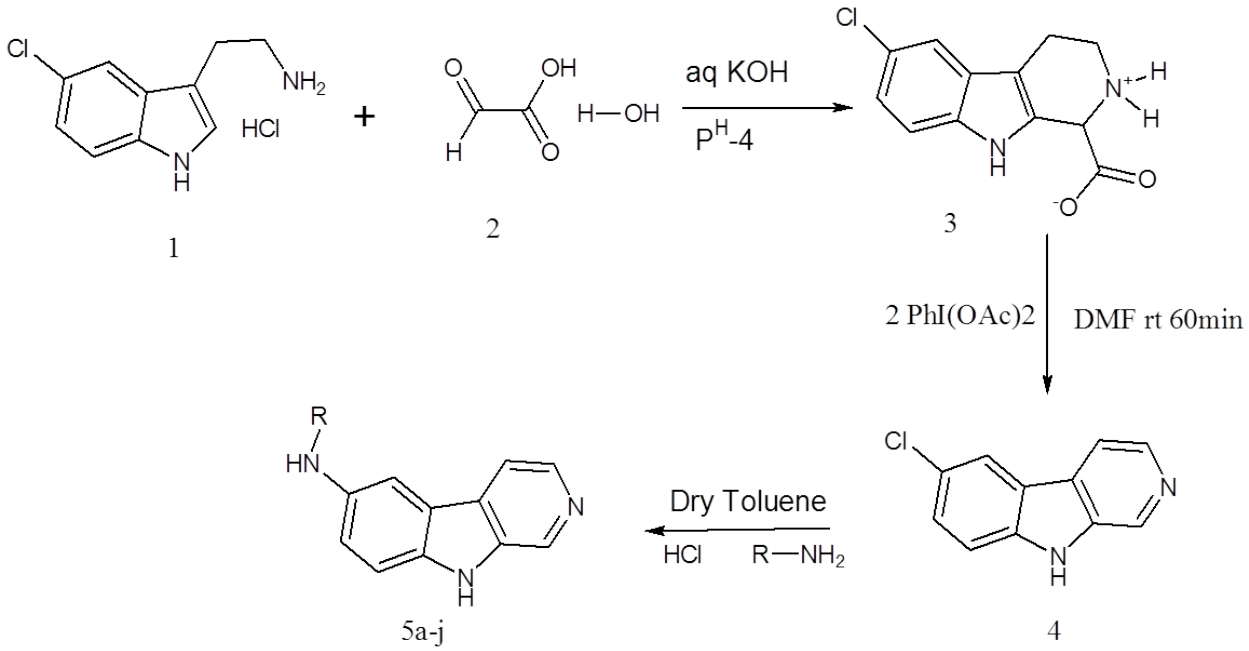
N-Methyl-9H-β-carbolin-6-amine (5a)

Yield 64.5%, Melting point 246^{o}C, Rf Value 0.42, Mol Formula $C_{12}H_{11}N_3$, Mol Weight 197.23. IR (Cm^{-1}) (KBr): 1197.64 (C=N); 3274.17 (NH). ^{1}H NMR (400MHz, DMSO): δ 2.48 (3H, s), 7.05-7.09 (1H, m), 7.25(1H, s), 7.54-7.58 (2H, m), 8.05 (1H, d), 8.68 (1H, s), 10.85 (1H,br s, NH). Mass m/z: 198 (M+1);

Table 1: Antibacterial activity of N-Substituted-9H- β-carbolin-6-aminederivatives (5a-j)

	Concentration in μg/ml Zone of inhibition (diameter in mm)																			
Com-pound	*S Pyogenes*				*B Subtilis*				*S Aureus*				*E Coli*				*P Aeruginosa*			
ml	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
5a	-	14	15	17	-	14	16	18	-	12	14	16	-	14	16	18	-	12	14	16
5b	-	14	16	17	-	13	16	19	-	12	14	17	-	13	16	18	-	11	14	16
5c	-	15	16	16	-	14	16	18	-	12	14	16	-	14	16	18	-	12	14	16
5d	-	14	15	16	-	14	17	18	-	12	14	16	-	14	17	18	-	13	14	16
5e	-	13	15	16	-	16	17	19	-	14	17	19	-	16	19	21	-	16	17	21
5f	-	13	15	16	-	14	15	15	-	12	13	14	-	14	15	16	-	11	13	14
5g	-	14	15	16	-	15	16	18	-	13	14	16	-	16	17	19	-	13	14	16
5h	-	15	16	18	-	17	18	20	-	15	16	18	-	17	21	22	-	15	17	19
5i	-	14	16	19	-	18	18	19	-	14	16	19	-	17	20	23	-	16	18	19
5j	-	15	16	20	-	15	18	20	-	13	16	19	-	17	20	23	-	14	18	20
Stan-dard	14	17	19	24	17	20	21	26	15	17	20	24	16	19	22	25	14	18	20	23
Con-trol)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Note: Standard-Amoxicillin, Control- DMSO, '-' denotesno activity.



Scheme 1: The synthetic route to compounds 5 a-j

N-Ethyl-9H–β-carbolin-6-amine (5b)

Yield 74.5%, Melting point 256^{o}C, Rf Value 0.72, Mol formula $C_{13}H_{13}N_3$, Mol Weight 211.26. IR (Cm^{-1}) (KBr): 1115.21 (C=N); 3425.72 (NH). ^{1}H NMR (400MHz, DMSO): δ 1.19 (3H, t), 2.86-2.91 (2H, m), 7.05-7.09 (1H, m), 7.25 (1H, s), 7.54-7.58 (2H, m), 8.05 (1H, d), 8.71 (1H, s), 10.15 (1H,br s, NH). Mass m/z : 212(M+1).

N-Propyl-9H- β -carbolin-6-amine (5c)

Yield 52%, Melting point 230^{o}C, Rf Value 0.65, Mol formula $C_{14}H_{15}N_3$, Mol Weight 225.2. IR (Cm^{-1}) (KBr): 3393.63 (NH); 1107.40 (C=N). ^{1}H NMR **(**400MHz, DMSO)**:** δ 1.24 (3H, t), 2.49-2.57 (2H, m), 3.55 (2H, t), 7.05-7.09 (1H, m), 7.25 (1H, s), 7.52-7.56 (2H, m), 8.21 (1H, d), 8.76 (1H, s) 10.63 (1H,br s, NH). Mass m/z **:** 226 (M+1).

N-(Propan-2-yl)-9H-β-carbolin-6-amine (5d)

Yield 74.5 %, Melting point 225^{o}C, Rf Value 0.72, Mol formula $C_{14}H_{15}N_3$, Mol Weight 225.2. IR (Cm^{-1}) (KBr): 3424.22 (NH); 1115.27 (C=N).^{1}H NMR (400MHz, DMSO): δ 1.10 (6H, d), 4.78-4.86 (1H, m), 7.05-7.09 (1H, m), 7.26 (1H, s), 7.52-7.56 (2H, m), 8.21 (1H, d), 8.77 (1H, s), 10.54 (1H,br s, NH). Mass m/z: 226 (M+1).

N-Butyl-9H- β-carbolin-6-amine (5e)

Yield 56%, Melting point 241^{o}C, Rf Value 0.76, Mol formula $C_{15}H_{17}N_3$, Mol Weight 239.3. ^{1}H NMR (400MHz, DMSO): δ 1.03 (3H, t), 1.29-1.36 (2H, m), 2.71-2.76 (2H, m), 3.56 (2H, t), 7.05-7.09 (1H, m), 7.26 (1H, s), 7.53-7.57 (2H, m), 8.22 (1H, d), 8.79 (1H, s), 10.55 (1H,br s, NH). Mass m/z **:** 240(M+1)

N-(2-Methylpropyl)-9H-β-carbolin-6-amine (5f)

Yield 59%, Melting point 236^{o}C, Rf Value 0.81, Mol formula $C_{15}H_{17}N_3$, Mol Weight 239.3. ^{1}H NMR (400MHz, DMSO): δ 1.13 (6H, d), 3.15-3.18 (1H, m), 3.78 (2H, d), 7.08-7.12 (1H, m), 7.24 (1H, s), 7.49-7.52 (2H, m), 8.22 (1H, d), 8.78 (1H, s), 10.85 (1H,br s, NH). Mass m/z: 240(M+1).

N-Phenyl-9H- β-carbolin-6-amine (5g)

Yield 88%, Melting point 235^{o}C, Rf Value 0.52, Mol formula $C_{17}H_{13}N_3$, Mol Weight 259.3. IR (Cm^{-1}) (KBr): 3449.77 (NH); 1094.96 (C=N). ^{1}H NMR (400MHz, DMSO): δ 7.04-7.08 (1H, m), 7.27 (1H, s), 7.32-7.34 (1H, m), 7.42 (2H, d), 7.70 (2H, d), 7.91-7.93 (2H, m), 8.22 (1H, d), 8.67 (1H, s), 10.15 (1H,br s, NH). Mass m/z : 260 (M+1).

N-(4-Nitrophenyl)- 9H- β -carbolin-6-amine (5h)

Yield 67%, Melting point 255^{o}C, Rf Value 0.61, Mol formula $C_{17}H_{12}N_4O_2$, Mol Weight 304.3. ^{1}H NMR (400MHz, DMSO): δ 7.08-7.12 (1H, m), 7.27 (1H, s), 7.34 (2H, d), 7.62-7.70 (2H, m), 8.28 (2H, d), 8.33 (1H, d), 8.67 (1H, s), 10.69 (1H,br s, NH). Mass m/z: 305(M+1).

N-(p-tolyl)-9H-β-carbolin-6-amine (5i)

Yield 58%, Melting point 285^{o}C, Rf Value 0.52, Mol formula $C_{18}H_{15}N_3$, Mol Weight 273.3. ^{1}H NMR (400MHz, DMSO): δ 3.15 (3H, s), 7.04-7.09 (1H, m), 7.26 (1H, s), 7.32 (2H, d), 7.40 (2H, d), 7.51-7.60 (2H, m), 8.16 (1H, d), 8.68 (1H, s), 10.56 (1H,br s, NH). Mass m/z: 274(M+1).

N-[(Furan-2-yl) methyl]-9H- β -carbolin-6-amine (5j)

Yield 69%, Melting point 257^{o}C, Rf Value 0.56, Mol formula $C_{16}H_{13}N_3O$, Mol Weight 263.3. ^{1}H NMR (400MHz, DMSO): δ 4.71 (2H, s), 6.15-6.20 (2H, m), 7.05-7.09 (1H, m), 7.26 (1H, s), 7.53-7.58 (2H, m), 7.82-7.85 (1H, m), 8.13 (1H, d), 8.67 (1H, s), 11.21 (1H,br s, NH). Mass m/z: 264(M+1).

Anti bacterial activity

The final derivatives were screened for their antibacterial activity against Gram +Ve bacteria (Steptococcus pyogenes, Bacillus subtilis and Staphylococcus aureus) and Gram –Ve bacteria (Escherichia coli and Pseudomonas aeruginosa) by the disc diffusion method at concentrations of 10, 20, 30 and 40μg/ml in DMSO.

Amoxicillin was used as standard drug and DMSO as a control. The zone of inhibition was measured after 24h incubation at 37^{0}C

RESULTS AND DISCUSSION

Chemistry

The synthetic route for the compounds 5 a-j is outlined in Scheme 1. The Pictet Spengler condensation of 5-Chlorotryptamine (1) with glyoxalic acid (2) afforded compound 3 in 52.6% yield. ^{1}H NMR confirmed it, the disappearance of indole C_2 hydrogen in compound 3 which was present in compound 1 and also confirmed by mass spectrum.

The Mass spectrum of compound 3 obtained the base peak coincided with the molecular ion (M^+ +1) at 251.

Oxidative decarboxylation of compound 3 with iodobenzene diacetate led to the 6-Chloro-9H-β–carboline (4) in 76.5% yield. By shifting of aliphatic hydrogen's (C_1, C_3 and C_4) particularly at C_1 to the aromatic region in ^{1}H NMR confirmed that oxidative decarboxylation has occurred, and in mass spectrum (Molecular weight: 202.63) base peak obtained at 203 (M+1).

Compound (4) was treated with different mono substituted amines gave the title compounds (5 a-J) in between 53-88% yields. All new derivatives were characterized by their spectroscopic data (FT-IR, ^{1}H NMR and Mass), which were given under the experimental section.

The IR spectra showed absorption bands characteristic for NH indole and C=N stretching in the range between 3449-3274Cm^{-1} and 1197-1094Cm^{-1}, respectively.

The NH signal at C_6 did not appear in ^{1}H NMR spectra. Due to it may be hidden with in the aromatic region, remaining all protons appeared at their respective regions. Mass spectra also confirmed the structures of 5a-j. All compounds showed the presence of a base peak at M+1.

For example, Compound 4 was treated with methylamine gave 5a in 64.5% yield. It was possible to confirm the structure of the 5a by proton NMR, due to the presence of the methyl group signals.

It was also showed the base peak at 198 (M+1) in mass spectra. Like this, structures of the remaining all derivatives (5_b-5_j) were confirmed.

Antibacterial activity

In the current work, a set of 10 new β-Carboline derivatives were prepared according to Scheme 1

and screened for their in vitro antibacterial activity, and the zone of inhibition was measured after 24h incubation at 37^0C. Results were given under the experimental section. (Table 1).

The results obtained in this study revealed that all new derivatives were inactive at 10μg/ml Compounds 5h, 5i and 5j possessed good activities in growth inhibition of E-coli.

Compound 5e showed moderate activity against *E-coli* and *P-Aeruginosa*. Remaining all compounds (including 5e, 5h, 5i and 5j) showed mild to moderate activity with all bacterial strains.

CONCLUSION

A new series of N-Substituted-9H-β-carboline-6-amine derivatives were obtained by Pictet- Spengler reaction followed by oxidative decarboxylation and amination, with good yields. Final derivatives were confirmed spectroscopically and screened for antibacterial activity against various pathogenic bacterial strains by disc diffusion method. Among all tested compounds, compound 5h, 5i and 5j were active displaying good activity against E-coli while compound 5e was found to exhibit moderate antibacterial activity against *E-coli* and *P-Aeruginosa*.

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Conflict of Interest

The authors declare no conflict of interest

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