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

Alivelu Samala*1,2, Srinivasa Murthy M3, Krishna Mohan Gottumukkala2
1Department of Pharmaceutical Chemistry, Holy Mary Institute of Technology and Science, Bogaram, Keesara, Hyderabad, Telangana, India
2Centre for Pharmaceutical Sciences, Inst of Science and Technology, JNT University Hyderabad, Kukatpally, Telangana, India
3Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical sciences, Near Ramoji Film City, Deshmukhi, Nalgonda, India

ABSTRACT

β-Carbolines is also known as nor-harmate. 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 heterocycles 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, 1H 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.

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), anticancer (Dighe et al., 2015), antioxidant (Pari et al.,...
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 glyoxylic acid as starting materials, characterization by spectroscopically (FT-IR, ¹H 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 spectrometer. ¹H NMR spectra were recorded in 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-9H-pyrido[3,4-b]indol-2-ium-1-carboxylate; 4. 6-Chloro- β-carboline; 5a-j. N-Substituted-9H-pyrido[3,4-b]indol-6-aminnes.

R: 5a. -CH2-ch 5b. -CH2CH2CH3 5c. -CH2CH2CH2CH3 5d.-CH2CH3; 5e. -CH2(CH2)2CH3; 5f. -CH2CH2CH3; 5g.-C6H5; 5h. -C6H4 (4-NO2); 5i. -C6H4 (4-CH3); 5j. – Furfuryl.

**Synthesis of 6-Chloro-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°C) and cooled to room temperature. Glyoxylic acid monohydrate (oxoacetic acid) 0.9ml (0.01 mol) was dissolved in 30ml of water. The glyoxylic 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°C, Rf Value 0.72, Mol formula C12H11N2ClO2, Mol Weight 250.68. ¹H 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-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°C, Rf Value 0.42, Mol Formula C12H13N3, Mol Weight 197.23. IR (Cm⁻¹) (KBr): 1197.64 (C=N); 3274.17 (NH). ¹H 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);

N-Ethyl-9H-β-carbolin-6-amine (5b)

Yield 74.5%, Melting point 256°C, Rf Value 0.72, Mol formula C13H15N3, Mol Weight 211.26. IR (Cm⁻¹) (KBr): 1115.21 (C=N); 3425.72 (NH). ¹H 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)
Table 1: Antibacterial activity of N-Substituted-9H- β-carbolin-6-aminederivatives (5a-j)

| Compound | Zone of inhibition (diameter in mm) | S Pyogenes | B Subtilis | S Aureus | E Coli | P Aeruginosa |
|----------|-------------------------------------|------------|------------|----------|--------|--------------|
|          | Concentration in μg/ml              |            |            |          |        |              |
|          | 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 | 16 | - | 13 | 16 | 18 | - | 11 | 14 | 16 |
| 5c       | - | 15 | 16 | 17 | - | 14 | 16 | 18 | - | 14 | 16 | 17 | - | 15 | 17 | 18 | - | 11 | 14 | 16 |
| 5d       | - | 14 | 15 | 16 | - | 14 | 17 | 18 | - | 12 | 14 | 17 | - | 16 | 18 | 19 | - | 11 | 15 | 17 |
| 5e       | - | 13 | 15 | 16 | - | 16 | 17 | 19 | - | 14 | 17 | 19 | - | 16 | 19 | 21 | - | 16 | 18 | 20 |
| 5f       | - | 13 | 15 | 16 | - | 14 | 15 | 15 | - | 13 | 16 | 19 | - | 17 | 22 | 23 | - | 14 | 16 | 20 |
| 5g       | - | 14 | 15 | 16 | - | 14 | 16 | 18 | - | 16 | 17 | 19 | - | 17 | 20 | 21 | - | 14 | 18 | 20 |
| 5h       | - | 15 | 16 | 18 | - | 17 | 18 | 20 | - | 15 | 16 | 18 | - | 17 | 22 | 23 | - | 15 | 17 | 19 |
| 5i       | - | 14 | 16 | 19 | - | 18 | 19 | 20 | - | 14 | 16 | 19 | - | 17 | 20 | 21 | - | 14 | 18 | 20 |
| 5j       | - | 15 | 16 | 20 | - | 15 | 18 | 20 | - | 13 | 16 | 19 | - | 17 | 20 | 23 | - | 14 | 18 | 20 |
| Standard | - | 14 | 17 | 19 | 24 | 17 | 20 | 21 | 26 | 15 | 17 | 20 | 24 | 16 | 19 | 22 | 25 | 14 | 18 | 20 | 23 |
| Control  | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

*Note: Standard-Amoxicillin, Control- DMSO, '-' denotes no activity.

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

Yield 52%, Melting point 230°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): \(\delta\) 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°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): \(\delta\) 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°C, Rf Value 0.76, Mol formula C_{15}H_{17}N_{3}; Mol Weight 239.3. \(^1\)H NMR (400MHz, DMSO): \(\delta\) 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), 7.62 (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)**

© International Journal of Research in Pharmaceutical Sciences
Chemistry

RESULTS AND DISCUSSION

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 its structure 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, C, and C) particularly at C₁ 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 to afford 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 3449-3274 Cm\(^{-1}\) and 1197-1094 Cm\(^{-1}\), respectively. The NH signal at C₀ did not appear in \(^1\)H NMR spectra. Due to it its presence, it could be hidden within 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 methyl-lactam, which showed 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 confirmed by mass spectrum at 198 (M+1) in mass spectra. Like this, structures of the remaining all derivatives (5a-5j) 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°C. Results were given under the experimental section. (Table 1).

The results obtained in this study revealed that all new derivatives were inactive at 10 μg/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 bacte-

N-Phenyl-9H-β-carboline-6-amine (5g)

Yield 67%, Melting point 255°C, RF Value 0.5, Mol formula C₁₇H₁₁N₃O, Mol Weight 304.3. \(^1\)H NMR (400MHz, DMSO): \(\delta\) 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: 266 (M+1).

N-(phenyl)-9H-β-carboline-6-amine (5f)

Yield 69%, Melting point 257°C, RF Value 0.5, Mol formula C₁₆H₁₁N₃O, Mol Weight 263.3. \(^1\)H NMR (400MHz, DMSO): \(\delta\) 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: 254 (M+1).

N-(4-Nitropheny)-9H-β-carboline-6-amine (5i)

Yield 69%, Melting point 257°C, RF Value 0.5, Mol formula C₁₆H₁₁N₃O, Mol Weight 239.3. \(^1\)H NMR (400MHz, DMSO): \(\delta\) 7.82-7.85 (1H, m), 8.13 (1H, d), 8.67 (1H, s), 10.85 (1H, br s, NH). Mass m/z: 240 (M+1).

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

Yield 59%, Melting point 236°C, RF Value 0.81, Mol formula C₁₅H₁₇N₃, Mol Weight 239.3. \(^1\)H NMR (400MHz, DMSO): \(\delta\) 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).

Anti bacterial activity

The final derivatives were screened for their antibacterial activity against Gram +Ve bacteria (Streptococcus 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 40 μg/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°C.

Chemistry
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.

ACKNOWLEDGEMENTS

The author is grateful to Principal and management of Holymary Institute of Technology and Science (College of Pharmacy) for providing research facilities and also thankful to Laila Impex industry for providing spectral data.

Conflict of Interest

The authors declare no conflict of interest

Funding Support

No funding

REFERENCES

Bernardo, C., Olival, A. C. D., Ribeiro, A., Rodrigues, L. M., Esteves, A. P., Campos, A. M. 2012. Synthesis of beta-carboline derivatives. 16th International Electronic Conference on Synthetic Organic Chemistry, pages 1–10.

Bi, W., Bi, Y., Xue, P., Zhang, Y., Gao, X., Wang, Z., Bi, L. 2011. A new class of β-carboline alkaloid-peptide conjugates with therapeutic efficacy in acute limb ischemia/reperfusion injury. European Journal of Medicinal Chemistry, 46(5):1453–1462.

Cao, R., Peng, W., Wang, Z., Xu, A. 2007. β-Carboline Alkaloids: Biochemical and Pharmacological Functions. Current Medicinal Chemistry, 14(4):479–500.

Costa, E. V., Pinheiro, M. L. B., Souza, A. D. L., De, Barison, A., Campos, E. R., Valdez, R. H., Nakamura, C. V. 2011. Trypanocidal Activity of Oxaaporphine and Pyrimidine-β-Carboline Alkaloids from the Branches of Annona foetida Mart. (Annonaceae). Molecules, (11):9714–9720.

Cox, E. D., Cook, J. M. 1995. The Pictet-Spengler condensation: a new direction for an old reaction. Chemical Reviews, 95(6):1797–1842.

Dighe, S. U., Khan, S., Soni, I., Jain, P., Shukla, S., Yadav, R., Sen, P., Meenan, S. M., Batra, S. 2015. Synthesis of β-Carboline-Based N-Heterocyclic Carbenes and Their Antiproliferative and Antimetastatic Activities against Human Breast Cancer Cells. Journal of Medicinal Chemistry, 58(8):3485–3499.

Lin, G., Wang, Y., Zhou, Q., Tang, W., Wang, J., Lu, T. 2010. A facile synthesis of 3-substituted 9H-pyrido [3, 4-b] indol-1 (2H)-one derivatives from 3-substituted β-carboline. Molecules, 15(8):5680–5691.

Liu, J., Jiang, X., Zhao, M., Zhang, X., Zheng, M., Peng, L., Peng, S. 2010. A Class of 3 S -2-Aminoacyltetrahydro-β-carboline-3-carboxylic Acids: Their Facile Synthesis, Inhibition for Platelet Activation, and High in Vivo Anti-Thrombotic Potency. Journal of Medicinal Chemistry, 53(8):3106–3116.

Pari, K., Sundari, C. S., Chandani, S., Balasubramanian, D. 2000. β-Carbolines That Accumulate in Human Tissues May Serve a Protective Role against Oxidative Stress. Journal of Biological Chemistry, 275(4):2455–2462.

Polanski, W., Reichmann, H., Gille, G. 2011. Stimulation, protection and regeneration of dopaminergic neurons by 9-methyl-β-carboline: a new anti-Parkinson drug? Expert Review of Neurotherapeutics, 11(6):845–860.

Ratsch, C. 2005. The encyclopedia of psychoactive plants, ethnopharmacology and its applications. Park Street Press.

Savariz, F. C., Formagio, A. S. N., Barbosa, V. A., Foglio, M. A., de Carvalho, J. E., Duarte, M. C. T., Filho, B. P. D., Sarragiotto, M. H. 2010. Synthesis, antitumor and antimicrobial activity of novel 1-substituted phenyl-3-[3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-carboline derivatives. Journal of the Brazilian Chemical Society, 21(2):288–298.

Valdez, R. H., Tonin, L. T. D., Ueda-Nakamura, T., Silva, S. O., Filho, B. P. D., Kaneshima, E. N., Nakamura, . ., V, C. 2012. In vitro and in vivo trypanocidal synergistic activity of N-butyl-1-(4-dimethylaminio) phenyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxamide associated with benznidazole. Antimicrobial agents and chemotherapy, 56(1):507–512.

Yao, K., Zhao, M., Zhang, X., Wang, Y., Li, L., Zheng, M., Peng, S. 2011. A class of oral N-[(1S, 3S)-1-methyl-1, 2, 3, 4-tetrahydro-β-carboline-3-carboxyl]-N’-(amino-acid-acyl) hydrazine: Discovery, synthesis, in vitro anti-platelet aggregation/in vivo anti-thrombotic evaluation and 3D
QSAR analysis. European journal of medicinal chemistry, 46(8):3237–3249.