Research Article [Kumar & Fazal, 3(8): Aug., 2012]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Synthesis and anti microbial activity of novel 5-substituted pyrrolo-β-carbolines

S. Senthil Kumar[1]* and M. I. Fazal Mohamed[2]

1, Department of Chemistry, Dhanalakshmi Srinivasan Institute of Research and Technology, Siruvachur, Perambalur, (TN) - India

2, P.G and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Khaja nagar, Tiruchirappalli, (TN) - India

Abstract

A series of 5-substituted pyrrolo β-carbolines have been synthesized via Bischler-Napieralski reaction. The structures of these compounds have been established by various analytical methods. All the compounds have been evaluated for anti microbial activity against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) , two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi(*Candida albicans* and *Aspergillus niger*) by Disc diffusion method. The results of anti-bacterial screening reveal that the compounds possess antibacterial activities to certain extent and significant antifungal activities.

Key-Words: Synthesis, pyrrolo-β-carbolines,Bischler-Napieralski reaction, Anti microbial activity, Disc diffusion method.

Introduction

Infectious diseases are one of the leading cause for death of mankind worldwide.During the past few decades , new infectious diseases have appeared and old ones previously thought to be controlled have reemerged[1] and thus, despite of many significant developments in the antimicrobial therapy, many problems remains to be solved for most of antimicrobial drugs available[2].Hence, discovery of novel antimicrobial agents with better pharmacological profile is still highly desirable.β-Carboline is a key pharmacophore present in a large number of natural tricyclic alkaloids, which can be found in numerous plants and animals, exhibiting potent biological activities[3-12]. The total synthesis of these substituted β-carbolines have attracted great attention.

The biological potential of β-carboline alkaloids and the importance of the search for new antimicrobial agents have led us to study this class of compounds.

Many papers have reported the synthesis and biological studies of β-carboline derivatives[13-19].Generally there are two ways to synthesize β-carbolines.One is through the Bischler-Napieralski reaction[20] and the other through Pictet-Spengler reaction[21]. We selected the Bischler-Napieralski cyclisation in our study.

To the best of our knowledge, all the synthesized β-carboline derivatives are novel. We report here in the preparation of novel 5-substituted pyrrolo β-carboline derivatives and their anti microbial activities.

Material and Methods

General

Unless otherwise specified, reagents were purchased from commercial suppliers and used without further purification.Melting points were determined with a digital melting point apparatus and are reported with out correction. Reaction progress was monitored using analytical thin layer chromatography (TLC).The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 500 ultra shield spectrometer using $CDCl_3$ as solvent (unless otherwise stated) and TMS as internal standard.

Synthesis

Recently, we have reported the synthesis of a series of novel 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-ones in good yields [22]. Here we would like to extend the work for designing β-carboline skeleton by using 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one **(1)** as a key intermediate. 5-amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one **(2)** was synthesized from 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one **(1)** by oxidation using KMnO4 in DMF.

*** Corresponding Author**
E.mail: senswaan@gmail.com

Synthesis of 5-amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one **(2)**

4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one (5.58 mmol) was dissolved in DMF (10 ml) in a round bottomed ,short necked flask.The solution was cooled to 0^{o}C and potassium permanganate(2.53 mmol) was added to the mixture and then left stirring overnight.The mixture was poured in to water to collect the top deposit. Recrystallization was done by using methanol.

Yield:76%,Brown solid, m.p 232 - 235^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.06 (–NH),8.02(-CONH), 2.04(-NH_2),7.08- 8.92 (H, indole ring),2.91 (-CH_2). ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.11(-CONH),101.2-135.33 (C, indole ring),40.12 (-CH_2), 91.05(C,pyrrole ring), 141.14(C-NH_2 , pyrrole ring).

General Procedure for the synthesis of amides of 5-Amino-4-(1H-indol-3-yl)-1,3-dihydro-pyrrol-2-one **3(a-f)**

The corresponding acid chlorides were added (1.37 mmol) to the solution of **(2)** (1.26 mmol) in CH_3CN (20 ml) .The resultant mixture was refluxed with triethyl amine (2 ml) at 90^{o}C for 4 Hrs in a 250 mL single-necked, round-bottomed flask. Evaporation to dryness under reduced pressure yielded crude solid residues. Pure compounds were obtained as brown solids by column chromatography using MeOH-$CHCl_3$ as eluent.

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-acetamide (3a)

Yield:71%,Brown solid, m.p 295 - 298^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.12 (–NH,indole ring),8.06(-CONH), 8.04(-CONH,pyrrole ring),7.21-8.74 (H,indole ring),3.12(-CH_2), 1.93(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.14(-CONH),167.27(-CONH,pyrrole ring),101.2-136.23 (C,indole ring), 94.13(C,pyrrole ring), 127.24(C-NH_2, pyrrole ring),39.37(-CH_2), 19.05(-CH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-benzamide (3b)

Yield:76%, Brown solid, m.p 251 - 254^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.07 (–NH,indole ring),8.06(-CONH), 8.03(-CONH,pyrrole ring),7.13-9.15 (H,indole ring), 7.37-8.11(H,phenyl ring),2.96 (-CH_2).^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.34(-CONH,pyrrole ring),164.82(-CONH),102.31-137.03(C,indole ring), 125.05-133.34(C,phenyl ring),94.17(C,pyrrole ring), 126.84(C-NH_2,pyrrole ring),40.04(-CH_2).

4-Hydroxy-N-[3-(1H-indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-benzamide (3c)

Yield:69%, Brown solid, m.p 255- 258^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.11 (–NH, indole ring),8.02(-CONH), 8.01(-CONH,pyrrole ring),7.08-9.22 (H,indole ring),6.79-7.91(H,phenyl ring),2.81 (-CH_2),5.02 (-OH) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.14(-CONH,pyrrole ring),163.87(-CONH),101.55-136.11 (C, indole ring),115.05-160.34(C,phenyl ring),93.17(C,pyrrole ring), 126.74(C-NH_2 of pyrrole ring),40.63 (-CH_2).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-methyl-benzamide (3d)

Yield:72%, Brown solid, m.p 286- 289^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH, indole ring),8.03(-CONH), 8.04(-CONH,pyrrole ring),7.13-9.41 (H, indole ring),7.19-7.91(H,phenyl ring),2.75 (-CH_2),2.31 (-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.03(-CONH, pyrrole ring),164.44(-CONH),102.23-135.41 (C, indole ring), 125.05-140.34(C,phenyl ring), 93.57(C,pyrrole ring), 126.62(C-NH_2 of pyrrole ring),40.11 (-CH_2), 20.82 (-CH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-methoxy-benzamide (3e)

Yield:75%, Brown solid, m.p 303- 306^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.02 (–NH, indole ring),8.02(-CONH), 8.03(-CONH, pyrrole ring),7.08-9.33 (H,indole ring),6.89-7.91(H,phenyl ring),2.78 (-CH_2),3.71(-OCH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.21(-CONH,pyrrole ring),164.29(-CONH),102.53-135.64 (C, indole ring), 125.05-165.34(C,phenyl ring), 93.45(C,pyrrole ring), 126.73(C-NH_2 of pyrrole ring),40.34 (-CH_2), 55.92 (-OCH_3).

N-[3-(1H-Indol-3-yl)-5-oxo-4,5-dihydro-1H-pyrrol-2-yl]-4-nitro-benzamide (3f)

Yield:73%, Brown solid m.p 294 - 297^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH, indole ring),8.02(-CONH), 8.04(-CONH, pyrrole ring),7.11-9.41 (H,indole ring), 8.19-8.41(H,phenyl ring),2.89 (-CH_2) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.54(-CONH,pyrrole ring),164.11(-CONH),102.43-136.17 (C, indole ring), 123.05-152.34(C,phenyl ring), 40.53 (-CH_2).

General Procedure for the synthesis of 5-substituted pyrrolo β-carbolines **4(a-f)**

To a solution of **3(a-f)** (0.8 mmol) in acetonitrile(20 ml),$POCl_3$(3ml) was added carefully in a drop wise manner.The resultant mixture was heated to reflux for 3 Hrs. Acetonitrile and $POCl_3$ were removed under vacuum and the residues obtained were purified by chromatography using silica gel as the solid support and then eluted with MeOH-$CHCl_3$.

5-methyl-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4a)

Yield:68%,Black solid m.p 311 - 314^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.08 (–NH),7.95(-CONH),7.24-7.63 (H, indole ring),3.45 (-CH_2),

2.52(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.34(-CONH), 102.28-134.63 (C, indole ring), 121.32- 154.31 (C,pyridine ring),35.32 (-CH_2), 15.85(-CH_3).

5-phenyl-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4b)

Yield:65%,Black solid, m.p 269 - 272^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.12 (–NH),8.21(-CONH),7.11-7.64 (H, indole ring),7.21-8.10(H, phenyl ring)3.37 (-CH_2) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.42(-CONH), 101.83-136.21 (C, indole ring),127.18-140.16 (C, phenyl ring) 121.15- 154.08 (C,pyridine ring),34.8 (-CH_2).

5-(4-hydroxyphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4c)

Yield:68%,Black solid, m.p 277 - 280^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.14 (–NH),8.13(-CONH),7.03-7.53 (H, indole ring),6.92-7.87(H, phenyl ring)3.42 (-CH_2),5.14(-OH) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 167.75 (-CONH), 102.79-135.14 (C, indole ring), 121.03- 154.12 (C,pyridine ring), 117.22- 156.12 (C,phenyl ring),35.32 (-CH_2).

5-(4-methylphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4d)

Yield:67%,Black solid, m.p 299 - 302^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.07 (–NH),8.04(-CONH),7.06-8.21 (H, indole ring), 7.02-7.76(H, phenyl ring),3.41 (-CH_2),2.37(-CH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.17 (-CONH), 102.34-135.16 (C, indole ring), 120.85- 154.31 (C,pyridine ring), 127.26- 137.45 (C,phenyl ring), 34.36 (-CH_2), 19.83(-CH_3)

5-(4-methoxyphenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4e)

Yield:65%,Black solid, m.p 319 - 322^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.11 (–NH),8.03(-CONH),7.04-7.57 (H, indole ring),6.76-7.73(H,phenyl ring),3.43 (-CH_2),3.72(-OCH_3) ^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.23(-CONH), 102.61-134.68 (C, indole ring),121.03-154.32(C, pyridine ring),114.37-160.45(C, phenyl ring), 35.35 (-CH_2), 56.37(-OCH_3)

5-(4-nitrophenyl)-6H-pyrrolo[3,4-b]-β-carbolin-2-one (4f)

Yield:67%,Black solid, m.p 313 - 316^{o}C

^{1}HNMR Spectra ($CDCl_3$): δ (ppm)10.10 (–NH),8.02(-CONH),7.03-7.58 (H, indole ring),8.18-8.30(H, phenyl ring)3.36 (-CH_2),^{13}CNMR Spectra ($CDCl_3$): δ (ppm) 168.13(-CONH), 101.85-136.45 (C, indole ring),120.64-154.16(C,pyridine ring),124.21-147.10(C,phenyl ring),35.71 (-CH_2).

Antimicrobial activity

The antimicrobial activity was evaluated using disc diffusion method[23] by measuring the zone of inhibition in mm.All newly synthesized compounds i.e. **4(a-f)** were screened in vitro for their antibacterial activity against two Gram positive strains(*Staphylococcus aureus* and *Bacillus subtilis*) , two Gram- negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*) at concentration of 25 µg/ml.Antifungal activity was tested against *Candida albicans* and *Aspergillus niger*.Ciprofloxacin(5 µg/disc) was used as a standard drug for antibacterial screening and Nystatin (100 units/disc) was used as a standard drug for antifungal screening.

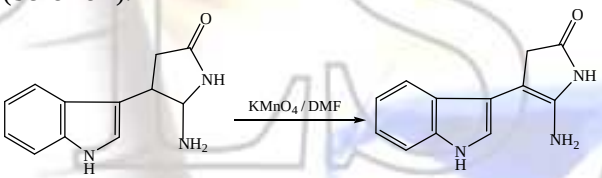
In all the determinations, tests were performed in triplicate and the average reading was taken.

Results and Discussion

Chemistry

In this paper, we describe a two-step preparation of 5-substituted pyrrolo β-carbolines **4(a-f)** using 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one **(2)** as the starting material. The later compound was prepared by the oxidation of 4-amino-5-(1H-indol-3-yl)-pyrrolidin-2-one **(1)** with $KMnO_4$ in the presence of DMF. (Scheme 1).

Starting from 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one **(2)** ,after reaction with various acid chlorides in the presence of acetonitrile and triethyl amine at refluxing,the corresponding amides obtained have been utilized as substrates in the cyclization step under Bischler-Napieralski reaction conditions.[24] (Scheme 2).



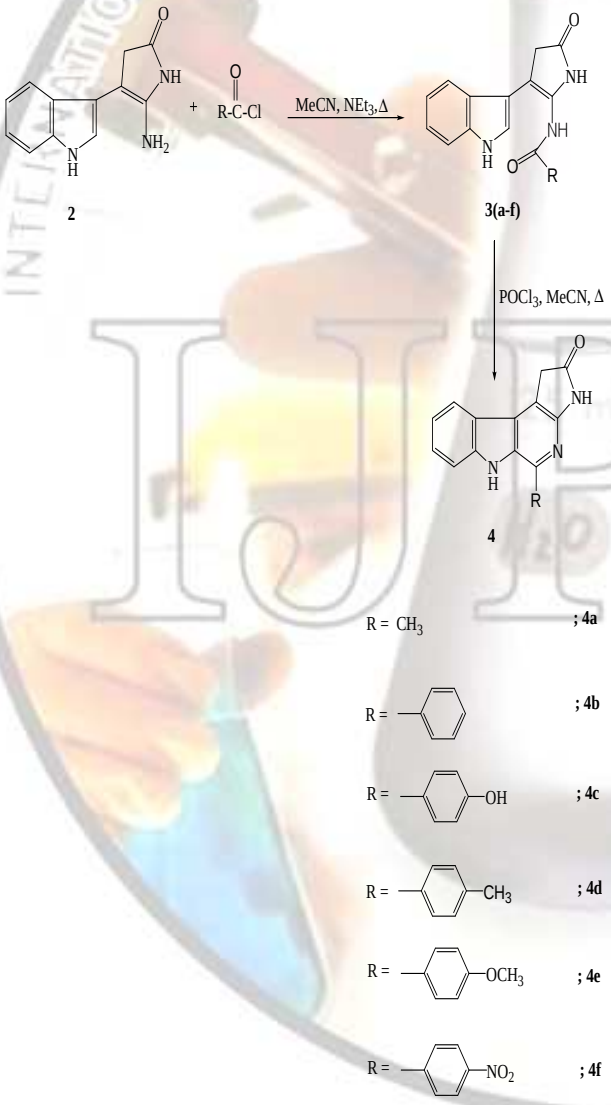
1 2

Scheme 1 : Synthesis of 5-amino-4-(1-*H*-indol-3-yl)-1,3-dihydro pyrrol-2-one

Antimicrobial activity

The results of the antimicrobial screening of 5-substituted- pyrrolo β-carbolines **4(a-f)** are shown in **Table 1**. According to the Table, all synthesized compounds exhibited moderate antibacterial activities and significant antifungal activities. Compound **4c** showed good activity against all tested Gram-positive and Gram-negative bacteria . It displayed much more potent activity against bacterial strains such as *Staphylococcus aureus* , *Escherichia coli* when compared with other compounds. Compound **4b** showed good activity against *Bacillus subtilis* and *Escherichia coli* when compared with other compounds. Compound **4a** showed good activity against *Pseudomonas aeruginosa*. Regarding the

activity of synthesized compounds against fungal strains again compound **4c** was found active against all tested fungi with good inhibiting zone value. Further compound iii showed equipotent antifungal activity against *Candida albicans,Aspergillus niger* as that of standard drug. Compound **4f** also showed equipotent antifungal activity against *Candida albicans* as that of standard drug.The rest of all compounds have shown good activities against *Candida albicans*. These results indicate that presence of hydroxyl group in compound **4c** is not only responsible for the antimicrobial activity, but also showing good effect indicating by greater inhibition zone.



Scheme 2 : Synthetic route for 5-substituted-pyrrolo-β-carbolines

Table 1: Antimicrobial activity of 5-substituted pyrrolo-β-carbolines (4a-4f) by disc diffusion method

Micro organisms	Values of diameters of inhibition zones in mm					
Gram positive bacteria	4a	4b	4c	4d	4e	4f
Staphylococcus aureus	20	20	23	22	20	20
Bacillus subtilis	12	19	14	10	14	16
Gram negative bacteria						
Escherichia coli	20	26	26	17	20	18
Pseudomonas aeruginosa	22	16	20	18	20	21
Fungi						
Candida albicans	20	18	23	20	20	22
Aspergillus niger	19	22	28	18	20	20

A thorough literature studies have left no room for doubt that all the synthesized β-carbolines are new to the literature. Antimicrobial activity evaluation reveal that among the tested compounds, compound **4c** shows significant inhibition , remaining compounds demonstrated potent to moderate antimicrobial activity. Hence it can be suggested that compound **4c** is a good candidate for further pharmacological studies to discover effective chemotherapeutic for the treatment of various infectious diseases caused by micro organisms.

Acknowledgement

The authors are grateful to the Head, SAIF,IIT-Madras for providing spectral and analytical data of the compounds. They are greatly acknowledge the support from Dept. of Microbiology, Periyar College of Pharmaceutical Sciences for Girls, Trichy for anti-microbial activity assessment.

References

1. Sharma P.C, Jain S, *Acta.Pharm.Sci.*,50,35(2008)
2. Sharma P.C, Jain S, *Acta.Pol.Pharm.*,65,551(2008)
3. Abramovitch R. A, Spenser I. D, *Adv. Het. Chem.*,3,79-207(1964)
4. Stuart K, Woo-Ming R, *Heterocycles*,3,223-264(1975)
5. Smith T. A, *Phytochemistry*,16,171-175(1977)
6. Allen J. R, *Phytochemistry*,19,1573-1582(1980)
7. Braestrup C, Nielsen M, Olsen C. E, *Proc. Natl. Acad. Sci. USA*,77,2288-2292(1980)
8. Schlecker W, Huth A, Ottow E, Mulzer J, *Synthesis*,1225-1227(1995)

9. Molina P, Fresneda P. M, *J.Chem. Soc., Perkin Trans.*,7,1819-1822(1988)
10. Molina P, Fresneda P. M, Zafra G. S, Almendros P, *Tetrahedron Lett*.,35,8851-8854(1994)
11. Dodd H. R, Ovannes C, Robert G, Potier P, *J.Med. Chem*.,32,1272-1276(1989)
12. Srivastava S. K, Agarwal A, Chauhan P. M. S, Agarwal S. K, Bhaduri A. P, Singh S. N, Fatima N, Chatterjee R. K, *J. Med. Chem*.,42,1667-1672(1999)
13. Shen Y.C, Chen C.Y, Hsieh P.W, Duh C.Y, Lin Y.M, Ko C.L, *Chem. Pharm. Bull*.,253, 32-36(2005)
14. Boursereau Y, Coldham I, *Bioorg. Med. Chem*.,14,5841-5844(2004)
15. Zhao M, Bi L, Wang W, Wang C, Baudy-Floc'h M, Ju J, Peng S, *Bioorg. Med. Chem*.,14,6998-7010(2006)
16. Chen Q, Chao R, Chen H, Hou X, Yan H, Zhou S, Peng W, Xu A, *Int. J. Cancer*.,114,675 682(2004)
17. Cao R, Chen Q, Hou X,Chen H, Guan H, Ma Y, Peng W, Xu A, *Bioorg. Med. Chem.*, 12,4613-4623(2004)
18. Cao R,Peng W, Chen H, Hou X, Guan H, Chen Q, Ma Y, Xu A, *Eur. J. Med. Chem*., 40,249-257(2005)
19. Cao R, Chen H, Peng W, Ma Y, Hou X, Guan H, Liu X, Xu A, *Eur. J. Med. Chem.*,40,991-1001(2005)
20. Lee, Jie J, *Name Reactions: A Collection of Detailed Mechanisms and Synthetic Applications*. Berlin, Heidelberg: Springer, (2007)
21. Whaley W.M and Govindachari T.R, *Org. Reactions*,6,151(1951)
22. Senthil Kumar S and Fazal Mohamed M.I, *Int.J.of Pharm. & Life Sci*.,2(12),1280-1286(2011)
23. Jeanne Moldenhauer, *Biopharm International*,18,34(2005)
24. Kayed A. Abu-Safieh, Mustafa M. El-Abadelah, Salim S. Sabri, Wolfgang Voelter, Cäcilia M. Mössmer, and Markus Stroebele,*Z. Naturforsch.*,57b,1327-1332(2002)