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A facile synthesis β-carbolines and studies on their antimicrobial activities

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ABSTRACT

Prepared 1-disubstituted and tetra cyclic β-Carbolines from easily available starting material tryptophan. The basic skeleton present in our isolated compounds is interesting, as some biologically active compounds possess this skeleton. The compounds are screened for their antibacterial activity.

KEYWORDS

β-carbolines;
Pictet-spengler reaction;
Anti-microbial activity;
2,3,4,9-Tetrahydro-β-carbolin-1ones;
Cyclization.

INTRODUCTION

β-Carboline is a common nucleus of various indole alkaloids[1], and many of these have a chiral center at its C-1 position. Thus, there have been several studies concerning the synthesis and biological activity of chiral 1-substituted 1,2,3,4-tetrahydro-β-Carboline derivatives and tetra cyclic β-carbolines using Pictet-Spengler reaction[2]. These β-carboline moiety is the core structure of various synthetic pharmaceuticals displaying a broad spectrum of biological activities such as hortiamine[3](hypotensive), canthins[4](cytotoxic and antileukemic activity), teldinafil[5](viagra series), ruta ecarpine[6](anti-inflammatory and cytotoxic) and several other β-carboline derivatives[7] show the anti HIV and antitumor activity.

As a continuation of our interest in the synthesis of biologically active molecules[8], we are herewith exploring a simple synthesis and anti-microbial screening of 1-substituted and tetra cyclic β-Carbolines. To the best of our knowledge, this is the first report, which explores the anti-microbial screening for these molecules. We report here a potentially significant simple route for the preparation of various biologically active molecules. It is worth mentioning that the basic skeleton present in our isolated compounds is interesting, as some biologically active compounds such as incasan[9], canthin[10], rutecarpine[11] possesses this skeleton.

RESULTS AND DISCUSSION

In the first instance, L-tryptophan is subjected to etherification using sulphuric acid as an acid catalyst in ethanol to yield light yellow color ethyl(2s)-2-amino-3-(1H-3-indolyl) propionate**(II)** with good yield. The ester is subjected to pictet-spengler reaction using different aldehydes to get the respective cyclized product. The ester is treated with propanaldehyde using triflouro acetic acid as an acid catalyst and refluxed for 12hrs at room temperature to yield ethyl 1-ethyl-2,3,4,9 tetra hydro-

1H-β-carboline-3-carboxylate **(1)** in 89% yield. Similarly, cyclization was tried with various aromatic aldehydes like benzaldehyde, which gives ethyl 1-phenyl-2, 3,4,9 tetrahydro-1H-β-carboline-3-carboxylate **(2)** in good overall yield. 4-hydroxy benzaldehyde was treated with ester using similar conditions and resulted in formation of ethyl 1-(4-hydroxyphenyl)-2,3,4,9 tetrahydro-1H-β-carboline-3-carboxylate **(3)** in good yield.

In continuation of our research on tetra cyclic β-carbolines, the ethyl 2,3,4,9-tetrahydro-1H--carboline-3-carboxylate**(III)** was treated with chloro acetyl chloride using potassium carbonate as a base in toluene medium and reaction mass was refluxed for 2hrs. The 2-methyl-1, 2,3,4,6,7,12,12a-octahydro pyrazino [1,2-b] β-carboline-1,4-dione**(4)** was isolated by column chromatography using ethyl acetate: pet ether as a mobile phase. The crude material is treated with methylamine in ethanol under reflux to yield brown compound **(4)**. Similarly, ethyl 2-(2-chloro acetyl) 2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylate (**IV**) is treated with butyl amine in ethanol to get brown color 2-butyl-1, 2,3,4,6,7,12,12a-octahydro pyrazino[1,2-b] -carboline-1,4-dione **(5)**. Ethyl 2,3,4,9-tetrahydro-1H-β-carbholine-3-carboxylate **(III)** is treated with 2-bromoethanol using potassium carbonate in ethanol under reflux for 24hrs to yield 3,4,6,7,12,12a-hexahydro1-H-(1,4) oxazino[4,3-b]-β-carboline-1-one**(6)**. The crude materials**(1-6)** were purified by column chromatography using silica gel(60-120mesh) and ethyl acetate: pet ether as a mobile phase. The general scheme can be depicted as SCHEME 1.

In vitro antibacterial assay

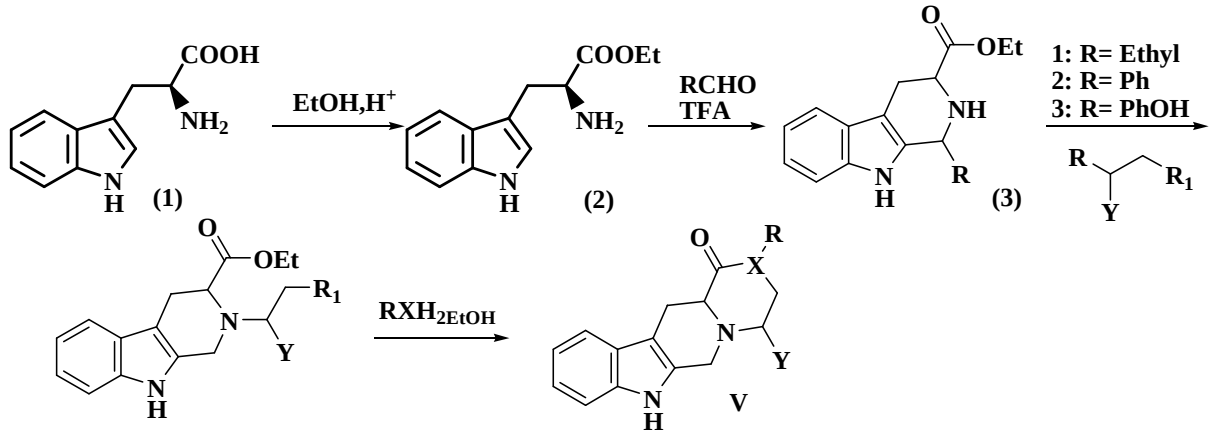
Organisms

The compounds were tested against a panel of organisms consisting of reference Gram positive and Gram negative bacteria. Both sensitive and resistant strains were included in the study. S.aureus ATCC 29213 Methicillin sensitive-MSSA, S.aureus ATCC 33591 Methicillin sensitive-MRSA, S.aureus DRCC446 Clinical isolate, E.faecalis ATCC 29212 Vancomycin sensitive, E.faecalis NCTC 12201 Vancomycin resistant strain, E.faecium NCTC 12202 Vancomycin resistant strain, H.influezae ATCC 49766 were used.

METHOD

Minimum inhibitory concentration(MIC) was determined by broth micro dilution method as per the guidelines of National committee of clinical laboratory standards(NCCLS). Doubling dilution of the test compound in the range of 32-1μg/ml were made using mueller hinton broth. The organisms were inoculated to obtain a final concentration of 5×10^4 to 1×10^5cfu/ml. The microtitre plates were incubated overnight in ambient air at 37^0C for 18-20hrs. For *H.influenzae*, Haemophilus test medium (HTM) was used.

Linezolid and Moxifloxacin were used as control antibiotics in the assay. Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial



SCHEME 1

agent that completely inhibits growth of the organism in micro dilution as well as detected by the unaided eye.

The reported compounds were tested for the *in vitro* antimicrobial assay and tested against a panel of organisms consisting of reference gram positive bacteria and gram negative bacteria. These compounds were found inactive. Linezolid and Moxifloxacin were used as control antibiotics in the assay.

EXPERIMENTAL

General methods

^{1}H NMR spectra were determined in CDCl3, DMSO-d_6 solution on Varian Gemini 200 MHz spectrometers. Proton chemical shifts(δ) are relative to tetra methyl silane(TMS, δ0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d(doublet), t(triplet) and m(multiplet). Coupling constants(J) are given in hertz. Melting points were determined using scientific capillary melting point apparatus and are uncorrected. Mass spectra were obtained on a HP-5989A mass spectrometer. Thin layer chromatography was performed on silica gel plates(SRL 230-400 mesh). All the solvents used were commercially available and was distilled before use.

Ethyl 1-ethyl-2,3,4,9 tetrahydro-1H-β-carboline-3-carboxylate (1)

3g(0.0129mmol) of ester**,** dichloromethane(30ml) and propanaldehyde(0.901g, 0.0155mmol) were taken into four-neck round-bottom flask. Triflouro acetic acid (0.73g, 0.0064mmol) was added slowly at room temperature. The reaction mass was maintained for 4hrs at room temperature. The progress of reaction was monitored by thin layer chromatography. The reaction mass was neutralized with saturated sodium bi carbonate solution. The solvent was evaporated and crude was purified by column chromatography using silica gel(60-120 mesh) using ethyl acetate: pet ether(50:50) as mobile phase to yield a dark yellow colour product in 89% yields.^{1}HNMR(200MHz, $CDCl_3$), δ10.03(s, NH), δ7.44(dd, 2H), δ7.30(dd, 2H), 4.28 (s, 2H), δ3.99(q, 2H), 3.72(t, 1H), δ3.04 (d, 2H), δ1.79 (m, 2H), δ1.07 (t, 3H), Anal.Calcd. For $C_{16}H_{20}N_2O_2$ (272); C, 70.56; H, 7.40; N, 10.29. Found: C, 70.62; H, 7.39; N, 10.32. MS(CI method) 273(M+1).

Ethyl 1-phenyl-2,3,4,9 tetrahydro-1H-β-carboline-3-carboxylate(2)

0.5g(0.0021mmol) of ester**,** dichloromethane(20ml) and benzaldehyde(0.265g, 0.0025mmol) were taken into four-neck round-bottom flask. Triflouro acetic acid (0.11g, 0.0025mmol) was added slowly at room temperature. The reaction mass was maintained for 6 hrs at room temperature. The progress of reaction was monitored by thin layer chromatography. The reaction mass was neutralized with saturated sodium bi carbonate solution. The solvent was evaporated and crude was purified by column chromatography using silica gel (60-120mesh) using ethyl acetate: pet ether(60:50) as mobile phase to yield a dark yellow color product in 89% yields. ^{1}HNMR(200 MHz, $CDCl_3$), δ7.37(m, 5H)δ5.24(s, 1H), δ3.93(q, 2H), δ3.37(t, 1H), δ3.18 (d, 2H), δ2.99(d, 2H), δ1.33(t, 3H), Anal. Calcd. For $C_{20}H_{20}N_2O_2$(320); C, 74.98; H, 6.29; N, 8.74. Found: C, 74.96; H, 6.32; N, 8.82. MS(CI method) 321 (M+1).

Ethyl 1-(4-hydroxyphenyl)-2,3,4,9 tetrahydro-1H-β-carboline-3-carboxylate (3)

0.5g(0.0021mmol) of ester**,** dichloromethane(20ml) and benzaldehyde(0.265g, 0.0025mmol) were taken into four-neck round-bottom flask. Triflouro acetic acid (0.11g, 0.0025 mmol) was added slowly at room temperature. The reaction mass was maintained for 6 hrs at room temperature. The progress of reaction was monitored by thin layer chromatography. The reaction mass was neutralized with saturated sodium bi carbonate solution. The solvent was evaporated and crude was purified by column chromatography using silica gel (60-120mesh) using ethyl acetate; pet ether (60:50) as mobile phase to yield a dark yellow color product in 89% yields. ^{1}HNMR(200 MHz, $CDCl_3$) δ 10.18 (s, NH), δ7.20(m, 5H),) δ5.49(s, 1H), δ 3.99 (q, 2H), δ 3.37(t, 1H), 3.18(d, 2H),δ3.04(d, 2H), δ1.08(t, 3H), Anal. Calcd. For $C_{20}H_{20}N_2O_2$(320); C, 74.98; H, 6.29; N, 8.74. Found: C, 74.96; H, 6.32; N, 8.82. MS (CI method) 321(M+1).

2-methyl-1,2,3,4,6,7,12,12a-octahydro pyrazino [1,2-b]β-carboline-1,4-dione(4)

120mg (0.0003mmol) of ethyl 2-(2-chloroacetyl)-2,3,4,9 tetrahydro-1H-β-carbolin-3-carboxylate**,** ethanol(5ml) and methylamine were taken into four-neck

round-bottom flask. The reaction mass was refluxed for 12hrs. The progress of reaction was monitored by thin layer chromatography. The solvent was evaporated and product was purified by column chromatography using silica gel (60-120 mesh) to yield a brown color product in good yields. ^{1}HNMR (200 MHz, $CDCl_3$), δ10.34(s, H), δ7.5-7.4 (m, ΦH), δ7.30-7.07(m, ΦH), δ 4.12(s, 2H), δ3.18(t, 2H), δ3.05(d, 2H), δ2.94 (m, 2H), δ2.58(s, 3H), Anal. Calcd. For $C_{15}H_{15}N_3O_2$ (269); C, 66.90; H, 5.61; N, 15.60. Found: C, 66.92; H, 5.72; N, 15.7. MS(CI method) 270(M+1).

2-butyl-1,2,3,4,6,7,12,12a-octahydro pyrazino[1,2-b]β-carboline-1,4-dione (5)

500mg (0.0015mmol) of ethyl 2-(2-chloroacetyl)-2,3,4,9 tetrahydro-1H-β-carbolin-3-carboxylate**,** ethanol (5ml) and butyl amine(125mg, 0.0017mmol) were taken into four-neck round-bottom flask. The reaction mass was refluxed for 14hrs. The progress of reaction was monitored by thin layer chromatography. The solvent was evaporated and product was purified by column chromatography using silica gel (60-120 mesh) to yield a brown color product in 72% yields. ^{1}HNMR(200MHz, $CDCl_3$) δ8.30 (s, NH), δ7.41 (dd, 2H), δ7.29(dd, 2H), 3.45(t, 2H), 3.40(s, 1H), δ3.30(t, 2H), δ3.20(t, 2H), δ1.55 (m, 2H), δ1.33 (m 2H) δ0.96(t, 3H), Anal. Calcd. For $C_{18}H_{23}N_3O$ (297); C, 66.90; H, 5.61; N, 15.60. Found: C, 66.92; H, 5.72; N, 15.7. MS (CI method) 298 (M+1).

3,4,6,7,12,12a-hexahydro1-H-(1,4) oxazino [4,3-b]-β-carboline-1-one(6)

1g(0.004mmol) of ethyl 2-(2-chloroacetyl)-2,3,4,9 tetrahydro-1H-β-carbolin-3-carboxylate**,** ethanol (20ml), potassium carbonate(1.1g, 0.008mmol) and 2-bromoethanol(0.563 g, 0.0045mmol) were taken into four-neck round-bottom flask. The reaction mass was refluxed for 24hrs. The progress of reaction was monitored by thin layer chromatography. The solvent was evaporated and product was purified by column chromatography using silica gel(60-120 mesh) to yield a brown colour product in 57% yields. ^{1}HNMR(200 MHz, $CDCl_3$), δ9.76(s, NH), δ7.43(dd, 2H), δ 7.31 (dd, 2H), 4.46(t, 2H), 4.05(s, 2H), δ3.34(t, 1H), δ3.30 (d, 2H), δ2.71(t, 2H), Anal. Calcd. For $C_{15}H_{15}N_3O_2$ (242); C, 69.41; H, 5.82; N, 11.56. Found: C, 69.52; H, 5.93; N, 11.50. MS (CI method) 243(M+1).

Thus, we have prepared 1-disubstituted and tetra cyclic β-Carbolines from easily available starting material tryptaphan by simple methodology. We explored the first anti-microbial screening study for these molecules. Many more β-Carboline derivatives are under preparation, which are precursors for the various biologically active molecules, will be communicated shortly.

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