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Synthesis and biological evaluation of β-carboline derivatives as an anxiolytic agents

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ABSTRACT

In our present research work, we have synthesized a series of β-carboline derivatives and evaluated for their anxiolytic activity. The structures of the compounds have been confirmed by IR and NMR spectroscopy. Newly synthesized compounds were tested for anxiolytic activity using elevated plus maze model. Among the synthesized compounds, N-(4-hydroxyphenyl)-9H-β-carboline-3-carboxamide (4a) was found to be most active with the maximum no. of entries in open arm and time spent in open arm.

Keywords: β-carboline, Anxiety, Anxiolytic agents, Synthesis

INTRODUCTION

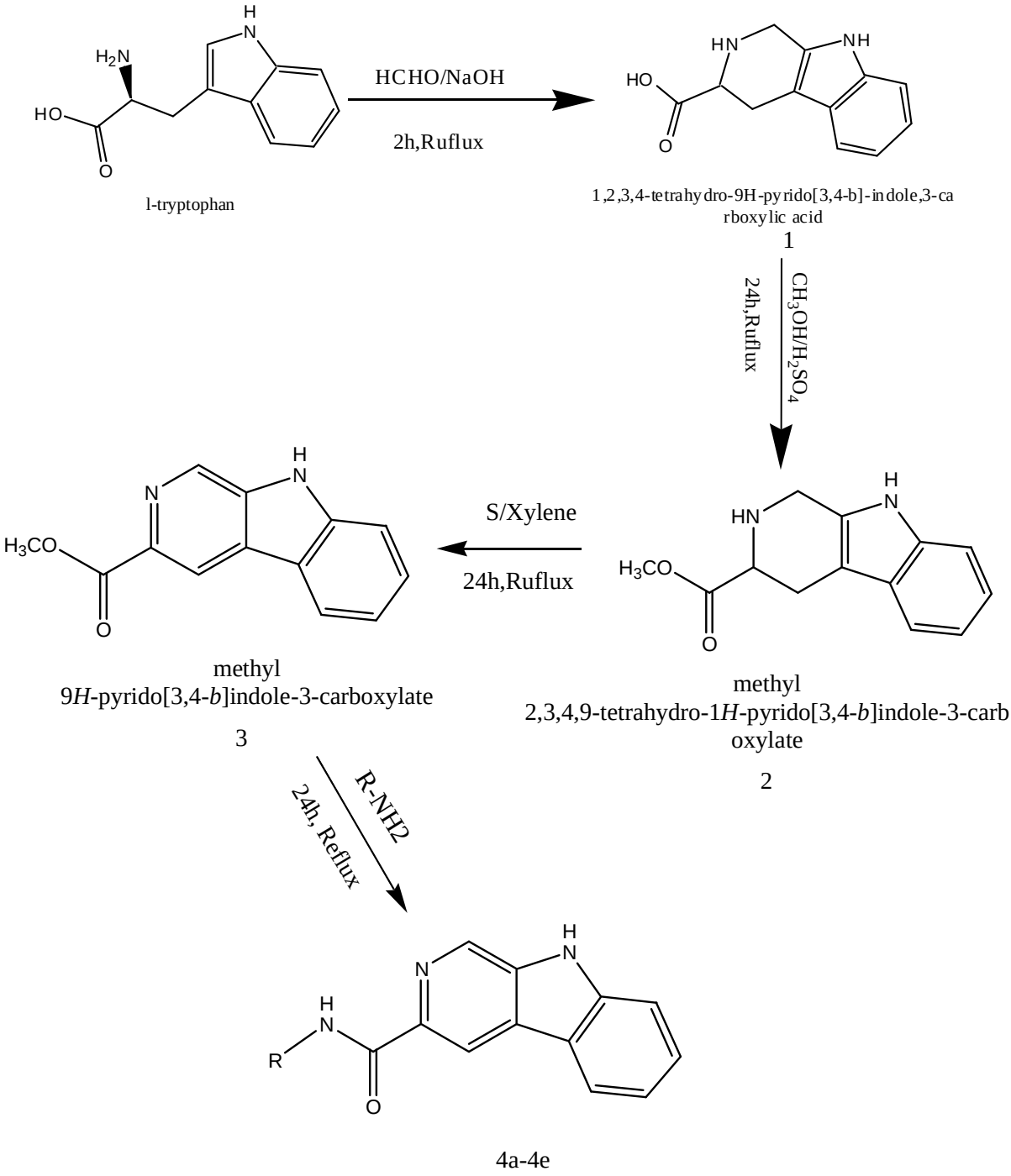
Anxiety is an emotional state characterized by disquietude of mind and a fearful anticipation of untoward events which may be brought on by stressful events in normal life.[1] Anxiety disorders have been classified according to the severity and duration of their symptoms and specific behavioural characteristics. Categories include: Generalized anxiety disorder (GAD), which is long lasting and low-grade, Panic disorder, which has more dramatic symptoms, Phobias, Obsessive-compulsive disorder (OCD)[2], Post-traumatic stress disorder (PTSD)[3], and Separation anxiety disorder (which is almost always seen in children).[4]

On the basis of benzodiazepines sites for ligand binding anxiolytic agents are classified as Benzodiazepine agonist, β-carboline Partial agonist-Abcarnil[5], β-carboline Inverse agonist, Benzodiazepine Antagonist.[6] However, the Benzodiazepines drugs possess undesirable side effects, such as Light-headness, Psychomotor and cognitive impairment**,** Vertigo**,** Confusional state**,** increased appetite**,** Weight gain**,** Alterations in sexual function. In response to these adverse effects, the development of new drugs to optimally manage anxiety has been strongly advocated. Thus the search for new anti anxiety drugs continues to be an active area of investigation in medicinal chemistry. A series of tetrahydro-β -carbolines, β-carbolines, and other nitrogen hetero cycles have prepared and evaluated them *in-vitro* with respect to their ability to bind to benzodiazepine receptors. The fully aromatic β-carbolines were more potent than their corresponding tetrahydro-β -carbolines derivatives. When substituent's possessing a carbonyl (CO_2Me, $COCH_3$, and CHO) was introduced at the β-Carboline-3-position the in vitro potency was augmented. The importance of the carbonyl moiety was further demonstrated when β-carboline-3-carboxylic acid was shown to bind tighter to benzodiazepine receptors at lower pH. A lower pH increases the concentration of the acid and decreases the concentration of the anion. [7]

In view of above, the objective of this investigation was the synthesis of some novel β-carboline derivatives and biological evaluation for their anxiolytic activity.

MATERIALS AND METHODS

The starting materials were commercially available and purchased from acros organics. Melting points were measured on a Veego Amp-1 melting point apparatus. Thin layer chromatography (TLC, silica gel-G) was used to monitor reactions and check product homogeneity. The structure of synthesized compounds was determined by spectral analysis. The λmax of synthesized compound was determined by using Shimadzu model 1700 spectrophotometer. IR spectra were recorded by Thermo Scientific Nicolet iS5 FTIR Spectrometer. ^{1}H-NMR spectra were recorded on a Burker Avance II- 400 MHz NMR spectrometer by using deuteriated chloroform ($CDCl_3$) as a solvent and TMS (Tetra Methyl Silane) as internal standard. Splitting patterns are described as singlet (s) and multiplet (m). (Chemical shifts in δ ppm).

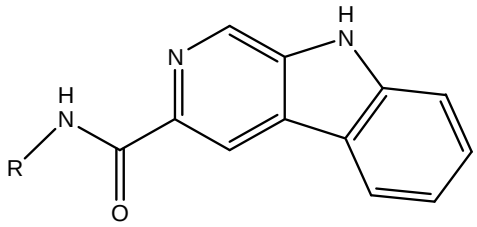


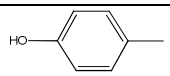
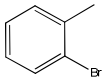
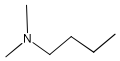
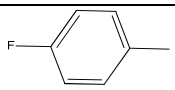
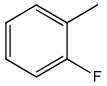
Scheme 1: Scheme of synthesis of β-carboline derivatives

Chemistry

Synthesis of β-carboline derivatives was done in four steps as shown in **scheme 1**. First step involves treatment of L-tryptophan with formaldehyde in Glacial acetic acid give β-carboline moiety. The second step involves the treatment of β-carboline with CH_3OH in the presence of H_2SO_4 as catalyst give esterified product methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate. The reduction of esetrified product methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate occurs in presence of xylene and sulphur. The reduced product methyl 9H-pyrido[3,4-b]indole-3-carboxylat reacted with different substituted anilines resulting the synthesis of different β-carboline derivatives. The list of synthesized compounds with their substitution is shown in table 1.

Table 1: The list of synthesized compounds with their substitutions



S. No.	Compound code	R	Molecular formula	Molecular Weight(gm)
1	4a		$C_{18}H_{13}N_3O_2$	303.10
2	4b		$C_{18}H_{12}BrN_3O$	365.02
3	4c		$C_{17}H_{21}N_4O$	297.17
4	4d		$C_{18}H_{12}FN_3O$	305.10
5	4e		$C_{18}H_{12}FN_3O$	305.10

Step-1: *Synthesis of 1,2,3,4-Tetrahydro-9H-pyrido[3,4-b]-indole,3-Carboxylic acids*

Equimolar portions of the appropriate Formaldehyde (3gm; 0.1mol) and L-tryptophan (20.409gm; 0.1mol) were dissolved in approximately 100mL of Glacial acetic acid. The mixture was refluxed for 2 hr. and then neutralized (pH 5) with NaOH and cooled. The precipitate was collected, washed well with H_2O, MeOH and dried in vacuum (20 mmHg, 100°C).[8]

Step-2: *Synthesis of methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate*

1,2,3,4-Tetrahydro-9H-pyrido[3,4-b]-indole, 3-Carboxylic acids (15.12gm, 70mmol) was suspended in 150mL of CH_3OH. A few drops of sulfuric acid were added to serve as a catalyst for the reaction.[9] The temperature was kept under 80°C. The mixture cleared slowly, and then a brown precipitate formed readily. The reaction was monitored by TLC (n-BuOH/ AcOH /H_2O, 4:l:l). The crystalline product was collected, washed sparingly with the corresponding alcohol, neutralized ($NaHCO_3$), stirred into H_2O (200 ml) and extracted with CH_2Cl_2 (10 x 200 ml). The combined CH_2Cl_2 extracts were dried.

Step-3: *Synthesis of methyl 9H-β-Carboline-3-carboxylate*

Methyl 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (9gm, 40mmol) was suspended in xylenes (150 ml). Sulfur (80mg) catalyst was added and the reaction mixture was stirred at 137°C for 24 hr, then cooled to freezing temperature and filtered through batsmen filter paper.[10] Brown shiny crystals were obtained.

Step-4: *Synthesis of 9H-β-Carboline-3-carboxamide derivatives*

Crystals of methyl 9H-pyrido[3,4-b]indole-3-carboxylate (1.35gm; 6mmol) in ethanol (50 mL) was mixed, and different anilines derivatives (6mmol) was added with continuous stirring. Then, refluxed the reaction mixture for 24hr. Cool the mixture at room temperature, precipitates was found. Precipitates were filtered and dried.

Synthesis of N-(4-hydroxyphenyl)-9H-β-Carboline-3-carboxamide (4a)
Yield 71.54%; m. p. 156-158 ^{0}C; λ_{max} 273.2nm; IR (ABS): 3338.61 cm^{-1} (N-H Amide), 3280 cm^{-1} (O-H), 3031 cm^{-1}(C-H), 1613 cm^{-1} (C=O, Amide), 1506 cm^{-1} (C=C), 1470cm^{-1} (C=N), 1253.84 cm^{-1} (C-O); ^{1}H NMR ($CDCl_3$): δ 4.97 (s, 1H, Ar-OH), 6.59-6.64 (d, 1H, Ar-NH), 7.24 (s, 4H, Ar-H).

Synthesis of N-(2-bromophenyl)-9H-β-Carboline-3-carboxamide (4b)
Yield 62.7%; m. p. 174-175 ^{0}C; λ_{max} 255nm; IR (ABS): 3343 cm^{-1} (N-H Amide), 3055 cm^{-1} (C-H), 1732 cm^{-1} (C=O, Amide), 1592.63cm^{-1} (N-H), 1488 cm^{-1} (C=N), 1421.4 cm^{-1} (C=C, Ar), 1172 cm^{-1} (C-Br), 1069 cm^{-1} (C-N), 743 cm^{-1} (Ar-H); ^{1}H NMR ($CDCl_3$): δ 6.54-6.56 (s, 2H, Ar-NH), 7.21-7.2(m, 10H, Ar-H).

Synthesis of N-(3-(dimethylamino)propyl)-9H-β-Carboline-3-carboxamide (4c)
Yield 57.9%; m.p. 258-260 ^{0}C; λ_{max} 256nm; IR (ABS): 3342.52 cm^{-1} (N-H Amide), 3056, 2925 cm^{-1} (C-H, Ar. Alkane), 1732.25 cm^{-1} (C=O, Amide), 1621 cm^{-1} (C-C), 1488.15 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 2.20(s, 6H, N-CH_3), 2.3-2.7(m, 6H, CH_2), 6.54-6.56(d, 2H, Ar-NH), 7.20-7.24(m, 6H,Ar-H).

Synthesis of N-(4-florophenyl)-9H- β-Carboline-3-carboxamide(4d)
Yield 48.3%; m.p. 195-196 ^{0}C; λ_{max} 263nm; IR (ABS): 3359.64 cm^{-1} (N-H Amide), 1557 cm^{-1} (C=N, Ar), 1416 cm^{-1} (C=C), 1335 cm^{-1} (C-F), 1105.79 cm^{-1} (C-N); ^{1}H NMR ($CDCl_3$): δ 6.5-6.9 (s, 2H, Ar-NH), 7.2-7.5 (m, 10H, Ar-H)

Synthesis of N-(2-fluorophenyl)-9H-β-Carboline-3-carboxamide(4e)
Yield 52.6%; m.p. 230-232 ^{0}C; λ_{max} 282.8nm; IR (ABS): 3344.68 cm^{-1} (N-H Amide), 1732 cm^{-1} (C=O), 1621 cm^{-1} (C-C), 1593 cm^{-1} (C=C, Ar), 1488 cm^{-1} (C=N), 1335 cm^{-1} (C-F), 1069 cm^{-1} (C-N), 743 cm^{-1} (C-H); ^{1}H NMR ($CDCl_3$): δ 6.5-6.9 (d, 2H, Ar-NH), 7.24 (m, 10H, Ar-H)

Anxiolytic Activity [11]
Anxiolytic Activity of synthesized compounds was done by using Elevated plus maze model. The Swiss albino mice of both sex (18-20gm) were procured. Animals were kept in clean dry cages a week before the beginning of the experiment to acclimatize with the experimental conditions and maintained for 7 days in the animal house of Faculty of Pharmacy, VNS Group of Institutions, Bhopal under standard conditions. i.e.; temperature: 24±10°C, relative humidity: 45-55 %, 12:12 h light: dark cycle. The animals were fed with standard pallet water *ad libitum*.

The swiss albino mice were weighed for the study. Dizepam suspension injection I.P. [3mg/kg body weight i.p.] was used as a reference drug applied over standard group animals (0.1ml/10gm body weight). Synthesized compounds [3mg/kg i.p.] suspension injected through intraperitoneal (i.p.) route in different test group animals (0.1ml/10mg body weight). Then anxiolytic activities were observed in both standard and test group of animals with the help of experimental model (Elevated plus maze). All the data of anxiolytic screening by Elevated plus maze method is summarized in table 2, which describe about the no. of entries in open arm and average time spent in open arm of anxious mice.

Table 2: Anxiolytic screening of some novel β-carboline derivatives

S. No.	Groups	Treatment	Dose	No. of entries in open arm	Average time spent in open arm (in second)
1.	Group 1	Control	(0.1ml/10mg) saline suspension	6.3±1.45	17.66±6.11
2.	Group 2	Diazepam	3 mg/kg	4.0±2*	73.6±26.244
3.	Group 3	4a	3 mg/kg	9.0±1.527*	81.66±21.058
4.	Group 4	4b	3 mg/kg	5.3±0.881	36±8.02
5.	Group 5	4c	3 mg/kg	4.3±1.452	36.3±16.271
6.	Group 6	4d	3 mg/kg	7.0±0.577	86.6±25.33
7.	Group 7	4e	3 mg/kg	5.6±0.33	48.6±4.807

*Values are represent mean ±SEM; n= 6 mice per groups; *P<0.05 as compared with control group*

RESULTS AND DISCUSSION

All these compounds were synthesized and characterized by melting point, thin layer chromatography, solubility study. The melting point of all the β-carboline derivatives ranges between 156-262°C. The thin layer chromatography of synthesized compounds was performed by using silica gel-G as adsorbent and Chloroform: methanol (8:2), Glacial acetic acid: methanol: water (8:2:1) as a solvent system. The result indicates that compounds are pure. The solubility study data represents that the synthesized compounds were insoluble in water, soluble in

Glacial acetic acid, and sparingly soluble in chloroform and DMSO which indicates that compounds are semi polar in nature.

The structure of synthesized compounds was determined by using UV, IR and NMR Spectroscopy. The UV spectroscopic data represents the λ_{max} (nm) values of the corresponding compounds. IR spectroscopy helps to identify the chemical structure of the compounds, like in the given data all the compounds show the peak values of the representing group which is present in the compounds. The ^{1}H NMR spectrum of synthesized compound 4a shows the peak at δ 7.28-7.5 indicated the presence of Aromatic H group and another peak at δ 4.29 explained the presence of hydroxyl group. The compound 4c shows peak at δ 2.7-2.3 and 2.20, explained the presence of CH_2 and CH_3 group respectively. The physicochemical and spectroscopic data support the laboratory synthesis of compounds.

After structural determination the *in-vivo* testing of the synthesized compounds also had been done by using **elevated plus maze** method in which swiss albino mice were used for animal model, Diazepam in a dose of 3mg/kg body weight, and for test compounds 3mg/kg body weight, method includes 6 groups on which this study was taken place as control, standard, and 5 test compound groups. No. of entries in open arm and time spent in open arm were represented as mean ± SEM.

The order of anxiolytic activity of the synthesized compounds is as follows:

4a > 4d> 4e > 4c>4b

Compound 4a showed maximum activity as compared to other compounds. The maximum activity shown by 4a may be due to hydroxyaniline at C-3 position of β-carboline ester. Synthesized compound 4a and standard drug diazepam showed 81.66±21.058 and 73.6±26.244 entries in open arm, it means these two compound show maximum anxiolytic activity as compare to control.

These result can be explore for further development of better anxiolytic agents.

CONCLUSION

β-carboline derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structure of the compounds thought of. The synthesized compounds showed a significant anxiolytic activity. Synthesized compounds increases the no. of entries and time spent in open arm of the animals, which indicated the decreases the anxiety in different group of animal in elevated plus maze model.

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