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Synthesis and antioxidant activity of novel 2-methyl-1-(2-morpholinoethyl)-indole-3-carboxylic acid analogues

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ABSTRACT

*The present study envisaged the discovery of novel antioxidant candidates using the indole incorporated morpholine scaffold. The structures of the final compounds were ascertained by IR, ^{1}HNMR, ^{13}CNMR and Mass analyses. All the final compounds screened in vitro antioxidant activity by DPPH method with ascorbic acid as positive control. Among the final derivatives,2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylicacid-3,4dimethoxyaniline (**IVb**) showed good antioxidant activity with an IC_{50} of 57.46µg/ml. It has been concluded that the presence of dimethoxyl group in the adjacent position in the aromatic region of the titled derivatives can contribute significant antioxidant activity.*

Key words: Indole, Antioxidant, DPPH, Fischer-Indole synthesis

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INTRODUCTION

Reactive oxygen species (ROS) and free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radicals can induce the oxidative damage of cell membranes, DNA, and proteins which are considered as the main reason in degenerative processes related to aging, cancer and atherosclerosis [1]. Free radicals are highly reactive chemical species possessing an unpaired electron formed by homolytic cleavage that can be considered as fragments of molecules [2]. Drugs possessing antioxidant and free radical scavenging activities are considered for the prevention or treatment of such diseases which are directly related to the lack of the antioxidant capacity of the body [3].Several indole derivatives have been reported indicating their antioxidant capacities [4-9]. The present study envisaged the development of novel antioxidant candidates using the indole scaffold by incorporating morpholinoethyl unit in the N1 position. The study highlighted the antioxidant effect of methoxyl group in various position of phenyl system in the titled derivatives.

MATERIALS AND METHODS

IR spectra were recorded on Shimadzu FT/IR spectrometer on KBr pellets recorded in cm^{-1} values.^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400mhz NMR spectrometer using DMSO as the solvent. Mass spectra were recorded on a JEOL GCmate mass spectrometer.

Synthesis of ethyl 2-methyl-1H-indole-3-carboxylate (I)

A mixture of ethylacetoacetate (6.3 ml, 0.05 mol) and glacial acetic acid (3 ml, 0.05 mol) are placed in the flat bottom flask and refluxed in methanol (25 ml) with the slow addition of phenyl hydrazine (5 ml, 0.05 mol) during first 1hr. After three hours, the reaction mixture poured into a 50 ml beaker and stirred vigorously while it solidifies. Then, sufficient water added and the solid filtered, dried and recrystallized with ethanol to obtain the compound **(I)**, ethyl 2-methyl-1*H*-indole-3-carboxylate.

Melting point: 180°C. IR (KBr) $\nu_{max}(cm^{-1})$: 3300.11 (Ar-H), 1451.43(R-COO-R), 1307.42 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.93(t, 3H, $OCH_2\underline{CH_3}$), 1.49 (s, 3H, Indole- $\underline{CH_3}$), 3.63(s, 1H, $\underline{NH}$), 4.09(m, 2H, CH_2), 7.19(m, 4H, Ar$\underline{CH}$). ^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm):14.47, 22.02, 43.89, 61.33, 122.10, 127.20, 128.26, 155.28, 164.82, 170.00, 181.08 MS: m/z $(M+1)^+$204.3

Synthesis of ethyl 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylate (II)

The compound **I** (1 g) dissolved in 10 ml of DMF and 4 eq of NaH with 4-(2-chloroethyl)morpholine. The mixture refluxed at 80 ^{0}C and the monitor on the TLC for the completion of the reaction. Then, water added and extracted with dichloromethane. Then dried on anhydrous sodium sulfate and evaporated to get the compound **(II)**, ethyl 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylate.

Melting point: 184°C. IR (KBr) $\nu_{max}(cm^{-1})$: 3300.18 (Ar-H), 1451.33(R-COO-R), 1307.45 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.92(t, 3H, $OCH_2\underline{CH_3}$), 1.33 (s, 3H, Indole- $\underline{CH_3}$), 1.49(t, 2H, =N-$\underline{CH_2}$), 2.33(t, 2H, $\underline{CH_2}$ morpholine), 3.60(t, 2H, $\underline{CH_2}$), 4.14(m, 2H, CH_2), 7.19(m, 4H, ArCH). ^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm):14.17, 53.43, 53.60, 57.61, 61.39, 66.83, 122.74, 127.67, 128.94, 131.31, 144.43, 170.64. MS: m/z $(M+2)^+$347.3

Synthesis of 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid (III)

Melting point: 176.5 °C. The compound **II** dissolved in methanol and stirred for 2 hours with 1eq of NaOH. The product extracted with dichloromethane and water. The aqueous layer collected and acidified with 1N. HCl to form the compound **(III)**, 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylic acid.

IR (KBr) $\nu_{max}(cm^{-1})$: 3300.18 (Ar-H), 1451.33(R-COO-R), 1307.45 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.94-0.96(t, 4H, CH_2), 1.43 (s, 3H, Indole- $\underline{CH_3}$), 3.36, (t, 4H, $\underline{CH_2}$ morpholine), 7.17(m, 4H, ArCH),11.08 (s, 1H, COOH)^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm): 11.98, 46.11, 53.68, 53.84, 66.91, 122.71, 127.34, 128.51, 131.01, 155.27, 173.71. MS: m/z $(M+2)^+$289.2

Synthesis of 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid - trimethoxy aniline (IV)

The compound **III** dissolved in dichloromethane, 1eq of dicyclohexylcarbodiimide+ and 1.5eq of butanol and kept the medium in ice bath. The corresponding aromatic amines added and stirred vigoursly yielded the final products.

2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid -3,4,5 trimethoxy aniline (IVa)

Melting point: 183.5°C. IR (KBr) $\nu_{max}(cm^{-1})$: 3337.26 (Ar-H), 1410.33(CO-NH), 1316.78 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.94-0.1.45(t, 4H, CH_2), 2.36 (s, 3H, Indole- $\underline{CH_3}$), 3.36-3.74 (s, 9H, $\underline{OCH_3}$), 6.60 (s, IH, NH), 6.51-7.17(m, 4H, ArCH),11.08 (s, 1H, COOH) ^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm): 15.57,24.69, 53.11, 53.44, 56.21, 57.61, 66.97, 106.04, 127.88, 129.09, 129.30, 131.02, 136.25, 144.60, 149.68, 172.61. MS: m/z $(M+2)^+$469.2

2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid-3,4 dimethoxy aniline (IVb)

Melting point: 186.2°C. IR (KBr) $\nu_{max}(cm^{-1})$: 3337.26 (Ar-H), 1410.33(CO-NH), 1316.78 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.94-0.1.45(t, 4H, CH_2), 2.34 (s, 3H, Indole- $\underline{CH_3}$), 2.44(d, 8H, CH_2) 3.36-3.74 (S, 6H, $\underline{OCH_3}$), 6.60 (s, IH, NH), 6.51-7.17(m, 4H, ArCH),11.08 (s, 1H, COOH) ^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm): 15.57, 24.69, 53.11, 53.44, 56.21, 57.61, 66.83, 114.94, 115.04, 106.04, 127.88, 129.09, 129.30, 131.02, 136.25, 144.60, 149.68, 172.61. MS: m/z $(M+2)^+$439.2

2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid-3, 5 dimethoxy aniline (IVb)

Melting point: 188°C. IR (KBr) $\nu_{max}(cm^{-1})$: 3391.97 (Ar-H), 1404.32(CO-NH), 1319.35 (C-N). ^{1}H NMR (DMSO, 400 MHz, δ ppm): 0.94-0.1.45(t, 4H, CH_2), 2.34 (s, 3H, Indole- $\underline{CH_3}$), 2.44(d, 8H, CH_2) 3.36-3.74 (S, 6H, $\underline{OCH_3}$), 6.60 (s, IH, NH), 6.51-7.17(m, 4H, ArCH),11.08 (s, 1H, COOH) ^{13}C-NMR (400 MHz, $CDCl_3$,δ ppm): 15.57, 24.69, 53.11, 53.44, 56.21, 57.61, 66.83, 114.94, 115.04, 106.04, 127.88, 129.09, 129.30, 131.02, 136.25, 144.60, 149.68, 172.61. MS: m/z $(M+2)^+$439.2

***In vitro* antioxidant activity by DPPH technique**

50µl of the solution with the pure compound will be added to 1mL of 100µM solution of DPPH. After 30 min of incubation at room temperature, the absorbance will be read against a blank at 517nm. Test compound concentration providing 50% inhibition (IC50) will be calculated from the graph plotting inhibition percentage against test compound concentration with ascorbic acid as positive control [10]

RESULTS AND DISCUSSION

The synthetic route for the formation of titled derivatives accomplished by four steps which is outlined in the figure 1. The first step involves the reaction between phenyl hydrazine and acetoacetic ester in presence of glacial acetic

acid medium afforded ethyl 2-methyl-1H-indole-3-carboxylate. Nucleophilic addition followed by a sigmatropic rearrangement is the basic principle involved in this reaction (Fischer-Indole synthesis) [11].The second step involves the substitution of 4-(2-chloroethyl)morpholine with the NH group of indole afforded ethyl 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylate. The third step involves the hydrolytic reaction of ethyl 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylate yielded 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylic acid. The final step involves the reaction between various substituted methoxy aniline and 2-methyl-1-(2-morpholinoethyl)-1*H*-indole-3-carboxylic acid afforded the corresponding amides.

The structures of the intermediates and final compounds were ascertained by spectral analysis. The appearance of IR band in the region of 1440, 1548 and 1316 indicates presence of CO, NH and C-N functional group of final derivatives. The appearance of a singlet peak in 11.08 δ of 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid (**III**) showed the full agreement of the hydrolytic process of ethyl 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylate (**II**). The disappearance of 11.08 δ signal from the compound III and the appearance of 11.08 δ singlet peak gave the full agreement for the formation of final amide system. The peaks obtained from the Mass spectra postulated with the molecular mass of the final structures.

All the final compounds evaluated for their *in vitro* antioxidant screening by DPPH method. The results of the antioxidant activity of final compounds with various concentrations are shown in the table 1. Among the final derivatives, 2-methyl-1-(2-morpholinoethyl)-1H-indole-3-carboxylic acid-3, 4 dimethoxyaniline (**IVb**) showed good antioxidant activity with an IC_{50} of 57.46µg/ml. It has been concluded that the presence of dimethoxyl group in the adjacent position in the aromatic region of the titled derivate can contribute significant antioxidant activity. In case of least active compound **IVc**, the methoxyl group are the 3rd and 5th position.The study can provide a new insight in the presence and position of methoxyl group in the aromatic system of the present scaffold toward free radical scavenging activity.

Table 1 Antioxidant activity of titled derivatives by DPPH method

Compound code	Concentration (µg)	Absorbance of control	Absorbance of test	Percentage inhibition	IC_{50}
IVa	20	1.24	1.12	9.66	97.85
	40	1.24	0.92	25.80	
	60	1.24	0.74	40.32	
	100	1.24	0.66	52.86	
IVb	20	1.53	1.28	16.34	57.46
	40	1.53	0.84	45.09	
	60	1.53	0.62	59.47	
	100	1.53	0.42	72.54	
IVc	20	1.36	1.30	4.41	135.17
	40	1.36	0.97	28.67	
	60	1.36	0.92	32.35	
	100	1.36	0.90	33.88	

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