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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF NEW BIS N'-(1H-BENZIMIDAZOL-2-YL)-N-ALKYLAMIDINE DERIVATIVES

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ABSTRACT

New series of Bis N'-(1H-benzimidazol-2-yl)-N-alkyl amidine derivatives **2a-e** were synthesized starting from (1H-benzimidazol-2-yl) iminoester **1** with two equivalents of ethane-1,2-diamine. The structures of compounds were elucidated by spectroscopic methods including IR, ^{1}H NMR, ^{13}C NMR, and ^{13}C NMR Dept 135° of **2b**, elemental analyses and mass spectral analysis. In the next step, the above mentioned compounds were screened for antioxidant activity. Their antioxidant activity was assessed using, the rapid and the most appropriate method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The results indicated that bis amidines **2a-e** tested possess good antioxidant activity. Compounds **2c** and **2e** showed excellent antioxidant activity, while compounds **2a**, **2d** and **2b** showed the lowest one.

Keywords: Benzimidazole, Bis amidines, DPPH method, ethane-1,2-diamine , Iminoesters.

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INTRODUCTION

Free radicals are among the main products of lipid oxidation and have been involved in over hundred diseases including cancer, atherosclerosis and arthritis[1]. Antioxidants are important compounds that reduce or neutralize the free radicals, thus protecting the cells from oxidative injury[2]. At present various research groups worldwide are directed towards the identification of new antioxidants to prevent radical-induced damage. The bis amidines, form an important class of compounds, are well known antiviral and antitumor[3]. Over the years, the bis amidines moiety have become an important class of heterocyclic compounds in organic synthesis due to their various biological properties. It is well known that bis amidines derivatives have therapeutic applications. Thus, there are various drugs incorporating in their structure used as antidegenerative [4] ,anti-inflammatory, antitumor[5], antimicrobial[6], antiparasitic[7], antibacterial[8], antiprotozoal[9], antimalarial [10], anticancer[11], anti HIV[12], antidegenerative[13], and urokinase inhibitor activities[14] are well documented in literature. In continuation of search on potent molecules exhibiting antioxidant activities we have synthesized a new series of of Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives and screened them for antioxidant activity which we wish to report in this paper.

2. MATERIALS AND METHODS

2.1. Materials

Iminoester **1** was synthesized and purified by a reported method[15]. All the chemicals were taken sigma Aldrich campany. Other chemicals used were of analytical reagent grade without further purification.

IR spectra were recorded with a Fourier Transform Infrared Spectrometer (FT- IR 200).^{1}H, ^{13}C NMR and ^{13}C NMR dept 135° spectra were recorded with $(CD_3)_2SO$ as the solvents containing TMS on a Brüker 300 MHz spectrometer (USA). The chemical shifts were reported in δ values relative to TMS (internal reference). For the ^{1}H NMR, the multiplicities of signals are indicated by the following abbreviations: s : singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Melting points (m.p.) were obtained using a Buchi melting point apparatus. Elemental microanalysis was performed on a Perkin - Elmer CHN-2400 analyzer apparatus. The electron spray ionization (ESI) positive MS spectra were recorded on a Brüker Daltonics LC–MS spectrometer (USA).

2.2. General procedure for synthesis of Bis amidine N'-(1H-benzimidazol-2-yl)-N-alkyl derivatives

Ethane-1.2-diamine (2 mmol) was added to a solution of iminoesters **1** (4 mmol) in ethanol (15 mL). The

reaction mixture was stirred and heated under reflux for 24 h. The solvent was removed under vacuum, and the resulting solid was filtered off, dried and crystallized from a mixture of chloroform and dioxane (v/v 7:3) to give (**2a-e).**

2.3 Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of bis amidines derivatives **2a-e** was evaluated using DPPH (1,1-diphenyl-2- picrylhydrazyl) colorimetric method[16]. Although a number of methods are available for determination of the antioxidant activity, the DPPH method is very common, rapid and has been shown to be one of the most appropriate methods[17, 18]. Different concentrations (5, 10, 20 and 40 µg/mL) of test compounds in dimethyl sulfoxide (DMSO) were prepared. In clean and labeled test tubes, the compound solution (1.0 mL) was added to the methanolic DPPH solution (2.0 mL, 0.1 mM) then the mixture was kept in the dark for 15 min, and the optical density was measured at 517nm using UV-Visible Spectrophotometer. The absorbance of the DPPH control was also noted. The percent scavenging activity was calculated using the following formula: inhibition (%) = 100*[($Abs_{control}$ -$Abs_{compound}$) /$Abs_{control}$]. The concentration of bis amidines derivatives providing 50% inhibition (IC_{50}) was calculated from the graph of the plot of inhibition percentage against bis amidines concentration (µg/mL)[19]. The synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

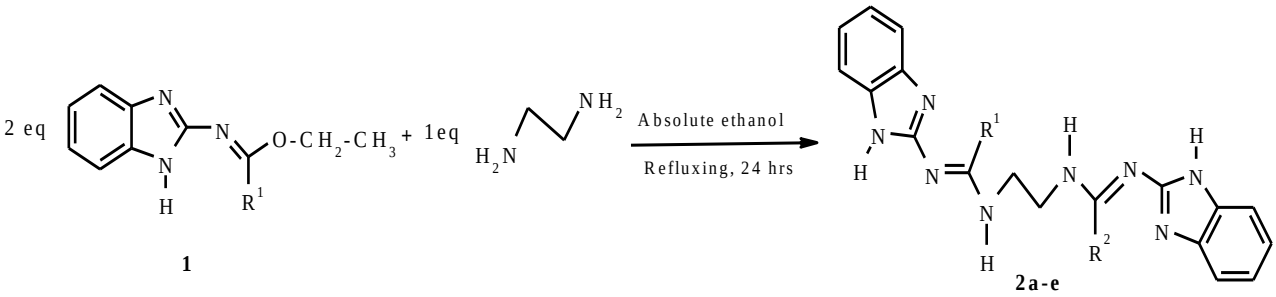
Statistical analysis

Statview v.5.0.1 software (SAS Institute, Cary, NC) was used for all statistical analyses. The significant difference of means were compared using analysis of variance (ANOVA) followed by Fisher's PLSD tests. All the analyses were carried out in triplicate measurements and mean values were used for the statistical analysis. Differences were considered statistically significant if $P< 0.05$.

3. RESULT AND DISCUSSION

3.1 General procedure for synthesis Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives

Treatment of (1H-benzimidazol-2-yl) iminoesters **1** with two equivalents of ethane-1,2-diamine under reflux of ethanol for 24h afforded the Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives **2a-e** in good yields (scheme1).



2a: R^1=R^2=CH_3, **2b:** R^1=R^2=CH_2-CH_3, **2c:** R^1=R^2= C_6H_5-, **2d:** R^1=CH_3, R^2=CH_2-CH_3, **2e:** R^1=CH_3, R^2=C_6H_5-

Scheme 1: Synthesis of Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives

The structures of all synthesized compounds **2a-e** were proven by IR, ^{1}H NMR, ^{13} C NMR, elemental analysis and MS spectral data. The formation of compounds **2a-e** was confirmed by the IR spectra showing a band in the region of 1626-1650 cm^{-1} assigned to the C=N groups and another band in the region 3440-3387 cm^{-1} corresponding to NH if benzimidazole moiety. The IR spectra of bis amidines derivatives **2a-h** exhibit a new absorption band at 3200-3150 cm^{-1} attributed to NH groups introduced by ethane-1,2-diamine.

The ^{1}H NMR spectrum of compounds **2a-e** revealed the disappearance of a signal specific to the ethoxy groups and the presence of new peaks assigned to the CH_2 moiety in the form of multiplets in the region δ 3.10 ppm- δ 3.90 ppm.

The ^{13}C NMR revealed the signal of the different carbons and confirmed the formation of compounds **2a-e**.The ^{13}C NMR dept 135° spectrum of compound **2b** gives positive signals (singlet) for CH_3 groups at δ 11.6 ppm - δ 12.2 ppm and gives negatives signals (singlet) for CH_2- group at δ 5.5 ppm-δ 29 ppm and δ 38 ppm-δ 41 ppm for CH_2 introduced by ethane-1,2-diamine. We can conclude that Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine **2a-e** are not symmetrical.

Interpretation

Compound 2a: Yield: 63%, m.p.: 270°C, IR (FT - IR 200, ν (cm^{-1})) ν = 3399 ($NH_{benzimidazole}$), ν = 3120 (NH), ν= 1632 (C=N).^{1}H NMR (DMSO-d6) δ ; 1.51 (s, 3H, C H_3), 1.32 (s, 3H, CH_3), 3.74 (m, 2H, CH_2), 3.82 (m, 2H, CH_2), 5.72 (broad s, 2H, NH), 6.91(board s, 2H, $NH_{benzimidazole}$), 6.25-7.27(m, 8$H_{aromatic}$).^{13}C NMR (DMSO-d6) δ ; 25.71, 176.13, 162.90, 46.25, 47.93, 168.32, 24.74, 161.05, 117.20- 143.63. Anal. Calcd.

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For $C_{20}H_{22}N_8$ (%): C, 64.15; H, 5.92; N, 29.93. Found: C, 64.20; H, 5.96; N, 29.95. ESI-MS $[M+1]^+$: *m/z* = 375

Compound 2b: Yield: 58%, m.p.: 266°C, IR (FT- IR 200, ν (cm^{-1})) ν =3410 ($NH_{benzimidazole}$), ν = 3145(NH), ν= 1635(C=N). ^1H NMR (DMSO-d6): δ; 2.46 (q, $^3J_{HH}$= 6.0 Hz, 2H, CH_2), 1.21 (t,$^3J_{HH}$ = 6.0 Hz, 3H, CH_3), 3.34 (m, 2H, CH_2), 3.48 (m, 2H, CH_2), 2.13 (q, $^3J_{HH}$= 6.0 Hz, 2H, CH_2), 1.09 (t, $^3J_{HH}$ = 6.0 Hz, 3H, CH_3), 7.32 (board s, 2H, $NH_{benzimidazole}$), 6.12 (board s, 2H, NH), 6.84-7.72 (m, $8H_{aromatic}$).^{13}C NMR (DMSO-d6): δ ; 12.22, 29.07, 174.23, 157.73, 38.36, 41.95, 166.71, 25.52, 11.63, 155.50, 111.81-138.72.^{13}C NMR dept 135° (DMSO- d6): δ ; 11.63, 12.22, 25.52, 29.07, 38.36, 41.95.Anal. Calcd. For $C_{22}H_{26}N_8$ (%): C, 65.67; H, 6.51; N, 27.84. Found: C, 65.70; H, 6.49; N, 27.82.ESI-MS $[M+1]^+$: *m/z* = 403.

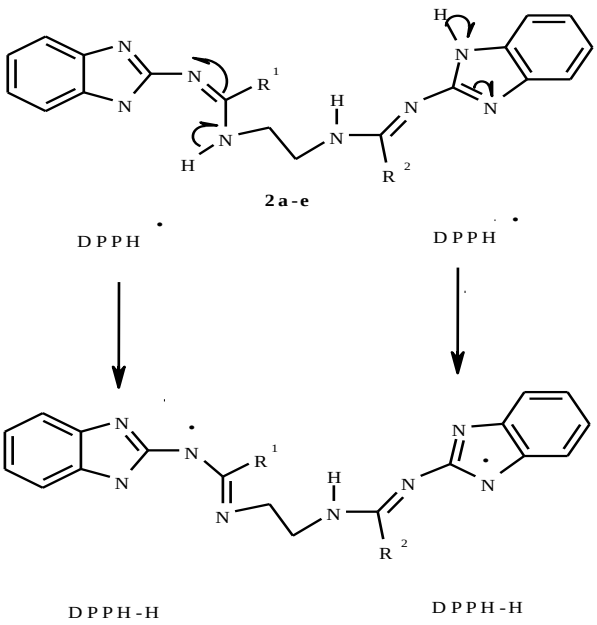
Compound 2c: Yield: 60%, m.p.: 280°C, IR (FT- IR 200, ν (cm-1)) ν = 3440 ($NH_{benzimidazole}$), ν = 3150 (NH), ν = 1650(C=N). ^1H NMR (DMSO-d6): δ; 3.75 (m, 2H, CH_2), 3.90 (m, 2H, CH2), 5.20 (board s, 2H, NH), 11.31 (board s, 2H, $NH_{benzimidazole}$), 7.33- 7.99 (m, 18 H $_{aromatic}$).^{13}C NMR (DMSO- d6): δ ; 156.70, 167.34, 36.23, 44.92, 164.80, 154.71, 109.80-143.84. Anal. Calcd. For $C_{30}H_{26}N_8$ (%): C, 72.27; H 5.26; N, 22.47. Found: C, 72.25; H, 5.27; N, 22.49. ESI-MS $[M+1]^+$: *m/z* = 499.

Compound 2d: Yield: 68%, m.p.: 240°C, IR (FT- IR 200, ν (cm-1)) ν = 3420 ($NH_{benzimidazole}$), ν = 3148 (NH), ν= 1626(C=N).^1H NMR (DMSO- d6): δ; 2.16 (s, 3H, CH_3), 1.18 (t,$^3J_{HH}$ = 9.0 Hz, 3H, CH_3), 2.67 (q, $^3J_{HH}$= 9.0 Hz, 2H, CH_2), 3.48 (m, 2H, CH_2), 3.27 (m, 2H, CH_2), 4.47 (board s, 2H, NH), 10.82 (board s, 2H, $NH_{benzimidazole}$), 6.84-7.80 (m, 8 $H_{aromatic}$).^{13}C NMR (DMSO- d6): δ ; 22.51, 161.95, 175.06, 37.82, 41.66, 173.45, 25.10, 9.98, 155.31, 111.40-138.46. Anal. Calcd. For $C_{21}H_{24}N_8$ (%): C, 64.93; H, 6.23; N, 28.84. Found: C, 64.91; H, 6.24; N 28.85. ESI-MS $[M+1]^+$: *m/z* = 389.

Compound 2e: Yield: 51%, m.p.: 230°C, IR (FT- IR 200, ν (cm-1)) ν= 3387($NH_{bnzimidazole}$), ν = 3130(NH), ν=1640 (C=N).^1H NMR (DMSO-d6): δ; 1.86 (s, 3H, CH_3), 3.46 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 5.42 (board s, 2H, NH), 10.60 (board s,2H, $NH_{benzimidazole}$), 6.24-7.25 (m, $13H_{aromatic}$).^{13}C NMR (DMSO- d6): δ22.40, 157.08, 163.78, 42.34, 43.64, 170.49, 155.11, 111.51- 137.90. Anal. Calcd. For $C_{25}H_{24}N_8$ (%): C, 68.79; H, 5.54; N, 25.67. Found: C, 68.80; H, 5.56; N, 25.69. ESI-MS $[M+1]^+$: *m/z* = 437.

3.2 Antioxidant activity

In this study, antioxidant activity of the synthesized compounds **2a-e** and the standard (BHT) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical assay (Scheme2)



Scheme 2: The probable mechanism for the reaction of compounds 2a-e with DPPH radical

Figure 1 shows free radical scavenging activity of Bis N'-(1H-benzimidazol-2-yl)-N-alkyl amidine der. at different concentrations, the radical scavenging activity was observed to increase with the dose of compound and standard

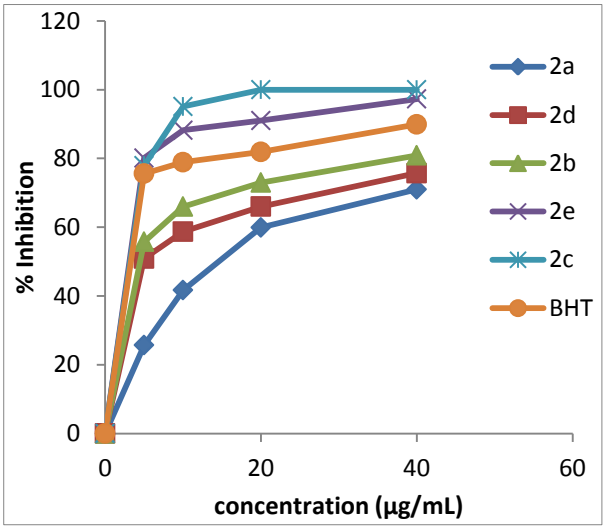
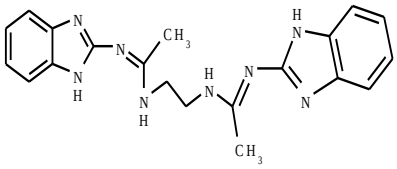
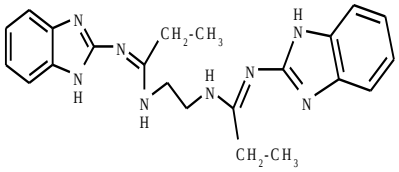
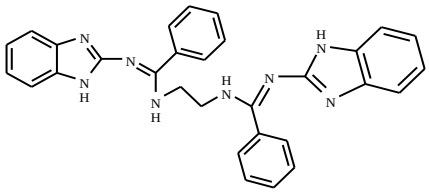
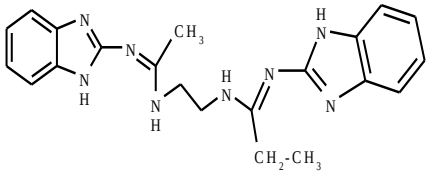
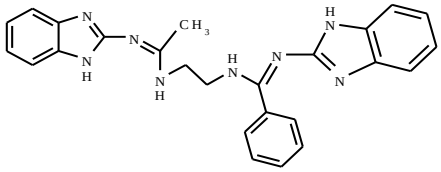


Figure 1: Scavenging activity of the synthesized Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives (2a-e) compared with BHT as standard

The IC_{50} values represent the concentration at which 50% of DPPH radical are inhibited. A lower IC_{50} value indicates a higher DPPH free radical scavenging activity. IC_{50} values of Bis amidine derivatives **2a-e** and the reference compounds are given in Table 1.

Table 1: Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives and their antioxidant activity

Entry	Structure	Compounds	DPPH Radical scavenging activity (IC50 µg/mL) (mean ± SEM)
1		**2a**	18.40* ± 0.15
2		**2b**	11.08* ± 0.12
3		**2c**	1.52* ± 0.03
4		**2d**	14.83* ± 0.11
5		**2e**	2.04* ± 0.02
6	BHT		4.87* ± 0.51

SEM – standard error of mean.
***significantly different ($p < 0.0001$)

According to our results, it is interesting to note that all the synthesized compounds **2a-e** displayed a radical scavenging activity by hydrogen donation (hydrogen benzimidazolic and hydrogen amidic). Indeed, **2c** and **2e** with phenyl group exhibited an antioxidant higher activity as suggested by its low IC_{50} (1. 52 and 2. 04 µg/mL, respectively).

Whereas, remaining compounds **2a, 2d** and **2b** are showing lower scavenging activity when compared to standard BHT. The introduction of methyl and ethyl group in the remaining compounds led to a considerable reduction of the radical scavenging activity as revealed by the significantly higher IC_{50} value (18.40µg/mL, 14.83µg/mL, 11.08 µg/mL respectively). These results indicate that the nature of substituent $\mathbf{R^1}$ and $\mathbf{R^2}$ in bis amidines moiety is very pivotal and affects the related radical scavenging activity. The scavenging effect of Bis N'-(1H-benzimidazol-2-yl)-N-alkyl amidine derivatives and the standard on the DPPH radical decreased in this order: **2c** >>**2e** >>**BHT**>>**2a**>>**2d**>>**2b**.

In the current study the Bis N'-(1H-benzimidazol-2-yl)-N-alkyl amidine derivatives were most efficient towards DPPH than drugs reported in other studies. In fact, *N*, *N'*-diphenylbenzamidine and *N*, *N'*-diphenyldodecamidine exhibited an antioxidant activity with an IC_{50}= 8.10 and 97,42 µg/mL respectively[20]. The maximum inhibition effectiveness rates of 2-methyl benzimidazole were 99.88% at 400 µg/mL[21]. Hydrazinecarbothioamides inhibited the DPPH activity with an IC_{50} = 39.39 µg/mL[22].

CONCLUSION

Five Bis N'-(1H-benzimidazol-2-yl)-N-alkylamidine derivatives **2a-e** were synthesized successfully and were subjected to investigation for their antioxidant activity *in vitro* towards DPPH. The results indicated the potential antioxidant properties of Bis amidines derivatives, and that the compound with phenyl group substituent on the **2c** and **2e** exhibited a significant radical scavenging activity on DPPH assay compared with the standard antioxidant BHT**.** These findings suggest that these molecules might serve as interesting compounds for the development of new antioxidant agents and might be used in many diseases.

Conflict of Interest

There is not any conflict of interest in this study.

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