Research Article

Synthesis and Biological Activities of 2-(α-p -substituted phenyl-α-benzimidazolo) methyl -1, 2, 3, 4-tetrazole

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ABSTRACT

In the present study, we described the synthesis and antimicrobial activities of 2-(α-p -substituted phenyl-α-benzimidazolo) methyl -1,2,3,4-tetrazole . The 2-(α-p -substituted phenyl-α-benzimidazolo) methyl -1,2,3,4-tetrazole (1b-3b) were synthesized by the reaction of 2-p-substituted phenyl-2-benzimidazolo acetonitriles(1a-3a), sodium azide ammonium chloride in DMF . The 2-p-substituted phenyl-2-benzimidazolo acetonitriles (1a-3a) were prepared by the reaction of benzimidazole , p-substituted benzaldehydes and Sodium cyanide. These compounds were characterized by IR , NMR and Mass spectroscopy. These synthesized (1b-3b) compounds showed both antibacterial and antifungal activities.

Keywords: Benzimidazole, tetrazole , acetonitrile, sodium azide.

INTRODUCTION

Tetrazole and its derivatives have attracted interest because of their unique structure and their applications as antihypertensive, anti-allergic, antibiotic and anticonvulsant agents.[1] The tetrazole functionality plays an important role in medicinal chemistry, primarily due to its ability to serve as the bioequivalent of the carboxylic acid group and also the class of tetrazole compounds has been used both as anticancer and antimicrobial agents. The importance of imidazoline and benzimidazloe , units arises, because they are found in many biologically active compounds[2-5]. In addition, the benzimidazole moiety is found in various synthetic pharmaceuticals displaying a broad spectrum of biological activity including anti-ulcer, anti-tumor and anti-viral effects[6-9]. Almost all benzimidazole derivatives with their two ring systems bear different functional substituent and this leads to essential modification of the physico-chemical, metabolic and pharmacokinetic properties of these drugs. Tissue selectivity of this type of antiulcer drugs is based on both their pH dependent accumulation, as weak bases in the acidic compartment of secreting parietal cell, and the subsequent acid-induced rearrangement of the parent compound to the pharmacologically active principle[10].

The addition of cyanide to imines (Strecker reaction) provides one of the most efficient methods for the synthesis of α-aminonitriles. α-Aminonitriles are important intermediates for the synthesis of amino acids[11] and various nitrogen containing heterocycles such as thiadiazoles and imidazoles[12] .The classical Strecker reaction is generally carried out with alkaline cyanides in aqueous solution. Among various cyanide ion sources,[13] trimethylsilyl cyanide is a safer and easily handled reagent compared to hydrogen cyanide, sodium cyanide, or potassium cyanide.

The present study deals with synthesis and biological activities of 2-(α-p -Substituted phenyl-α-benzimidazolo) methyl -1,2,3,4-tetrazole.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. IR spectra (K Br) were recorded on a Perkin Elmer 1800(FTIR) spectrometer. PMR spectra (DMSO- d_6) on a Varian EM-390 spectrometer using TMS as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Jeol JMS-D 300 Mass spectrometer operating at 70 eV. The purity of the compounds was confirmed by TLC using silica gel G. For TLC, Merck silica gel 60 G plate was used. The necessary chemicals were obtained from Merck and Fluka. All compounds showed satisfactory elemental analyses.

Antimicrobial Activity

The synthesized compounds in the present investigation have been tested for antimicrobial activity by well diffusion method. The organisms selected for the antifungal activity was carried out by using *Aspergillus niger, Candida albicans and Candida tropicalis*. The organisms selected for the antibacterial activity was carried out by using *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The plates are prepared as per the standard methods [14].

Antimicrobial assay

Antibacterial analysis was followed using standard agar well diffusion method to study the antibacterial activity of compounds .Each bacterial and fungal isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^5 colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 30 µL (5µg compound in 500 µL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 h at 37°C for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Chloramphenicol was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent.

1. Synthesis of 2-p-substituted phenyl-2-benzimidazoloacetonitriles (1a-3a)

1.1 Synthesis of of 2-p-Hydroxyphenyl-2-benzimidazoloacetonitrile (1a)

A mixture of 0.005mol p-Hydroxybenzaldehyde , 0.005mol benzimidazole and 0.0075mol trimethylsillylcyanide in 10 ml acetonitrile in the presence of 0.0005mol bismuth tri chloride was stirred at room temperature for 12hrs. After completion of the reaction, the reaction mixture was partitioned between 10 ml of ether and 50ml of water. The organic layer was washed with 50 ml brine, dried over sodium sulphate and concentrated. The crude was recrystallized from benzene-petroleum ether mixture. The pure compound melted at 192-193 ^{0}C.

1.2 Synthesis of 2--p-Anisyl-2-benzimidazoloacetonitrile (2a)

2-p-Anisyl-2-benzimidazoloacetonitrile (2a) was synthesized by using p-Anisaldehyde. The crude was recrystallized from benzene and the pure compound melted at 163-164 ^{0}C.

1.3 Synthesis of 2-p –N, N'-Dimethyl anilino -2-benzimidazoloacetonitrile(3a)

The 2-p –N,N'-dimethylanilino -2-benzimidazoloacetonitrile(3a) was synthesized by using p –N, N'-dimethylaminobenzaldehyde The crude was recrystallized from benzene-petroleum ether mixture . The pure compound melted at 137-138 ^{0}C.

2. Synthesis of 2-(α-p -Substituted phenyl-α-benzimidazolo)methyl-1,2,3,4- tetrazole(1b-3b)

2.1. 2-(α-p Hydroxyphenyl -α – benzimidazolo) methyl -1,2,3,4- tetrazole(1b)

A mixture of 2-p-Hydroxyphenyl-2-benzimidazoloacetonitrile (4.66g; 0.0 2mol) and Sodium azide (2.0 g; 0.02 mol) and Ammonium chloride (10.6 g; 0.2 mol) in 20 mL of DMF was taken in a 100 mL round bottomed flask. The contents were heated for 7 hours in oil bath maintained at 125^{0}C. The contents poured in ice water and acidified with HCl at pH is 2. .The tetrazole was filtered, washed with excess of water and dried. It was recrystallized from benzene and the compound melting at 150 ^{0}C .

Infra Red Spectral Data (KBr), λ values in cm $^{-1}$

3775(w) 3448 (w) 3083 (m) 2924 (m) 2854 (m) 2743 (s) 2666(w) 2583 (w) 1833(w) 1699 (s) 1632 (w) 1571 (s) 1472 (s) 1420 (s) 1410 (w) 1393 (m) 1301 (m) 1288 (m) 1250 (s) 1203 (w) 1168 (w) 1135 (w) 1049 (w) 1023 (w) 928 (w) 852 (m) 756 (m) 615 (w) 570 (m) 449 (w).

Proton Magnetic Resonance Spectral Data ($CDCl_3$ / TMS), δ in ppm

4.6	s	1H	C-H methine
6.7 -7.3	m	12H	Aromatic protons
7.8	s	1H	- OH phenolic
8.1	s	1H	C –H benzimidazole

Mass Spectral Values; m/z and %

343 (20) 342 (10) 341 (25) 324 (18) 323 (40) 297 (12) 286 (15) 256 (8) 248 (100) 236 (16) 224 (65) 222(20) 205 (28) 180 (10) 178 (24) 168 (25) 158(24) 156 (36) 150 (30) 149 (6) 132 (12) 131 (20) 127 (18) 120 (40) 119 (45) 118 (60) 117 (30) 105(15) 92 (35) 91 (10) 90(28) 63 (44)

Elemental Analysis		**C%**	**H%**
C_{21} H $_{15}$ N_3 O_2	Calculated :	73.9	4.39
(M.W . 341)	found :	72.5	4.23

2.2. Synthesis of 2-(α-p Anisyl-α – benzimidazolo) methyl -1,2,3,4-tetrazole (2b)

A mixture of 2-p-Anisyl-2–benzimidazoloacetonitrile (0.02mol) and Sodium azide (0.02 mol) and Ammonium chloride (10.6 g; 0.2 mol) in 20 mL of DMF was taken in a 100 mL round bottomed flask. The contents were heated for 7 hours in oil bath maintained at 130^{0}C. The contents poured in ice water and acidified with HCl at pH is 3. .The tetrazole was filtered, washed with excess of water and dried. It was recrystallised from benzene. The pure sample melting at 139^{0}C.

Infra Red Spectral Data (KBr), λ values in cm $^{-1}$

3416 (m) 3376 (m) 3066(w) 2949 (w) 2925 (m)2853 (m) 2799 (w) 2449 (w) 1783 (m) 1742 (s) 1620 (s) 1580 (s) 1480(s) 1397 (w) 1295 (m) 1252 (s) 1172(m) 1132 (w) 1026 (m) 926 (w) 850 (w) 831(w) 753 (m) 704 (w) 634(w) 567 (m) 450(m)

Proton Magnetic Resonance Spectral Data ($CDCl_3$ / TMS), δ in ppm

3.9	s	3H	-OCH_3
4.6	s	1H	C-H methine
6.7 -7.7	m	12H	Aromatic protons
8.0	s	1H	C –H benzimidazole

Mass Spectral Values ; m/z and %

355 (40) 354 (18) 324 (28) 285 (12) 265 (35) 263 (42) 248 (36)238 (55) 236(45) 158 (40) 156 (20) 146(25) 144(16) 129 (38) 119 (20) 118 (28) 117 (30) 107(18) 92 (50) 91 (100) 90(28) 63 (35)

Elemental Analysis		**C%**	**H%**
C_{22} H $_{17}$ N_3 O	Calculated:	74.36	4.78
(M.W . 355)	found :	73.52	4.52

2.3 2-(α-p –N, N'-Dimethylanilino -α – benzimidazolo) methyl -1,2,3,4-tetrazole(3b)

The 2-p –N,N'-dimethylanilino -2-benzimidazoloacetonitrile(3a) (0.02 mol) and Sodium azide (0.02 mol) and Ammonium chloride (10.6 g; 0.2 mol) in 20 mL of DMF was taken in a 100 mL round bottomed flask. The contents were heated for 7 hours in oil bath maintained at 130^{0}C. The contents poured in ice water and acidified with HCl at pH is 3. .The tetrazole was filtered, washed with excess of water and dried. It was recrystallized from benzene –petroleum ether mixture. The pure sample melted at 106^{0}C.

Infra Red Spectral Data (KBr), λ values in cm $^{-1}$

3416 (m) 3382(w) 3066(m) 2925 (w) 2852 (w) 2500 (m) 1850 (w) 1750 (w) 1670 (m) 1620 (s) 1603 (w) 1589 (w) 1516 (m) 1466 (w) 1420(m) 1409 (s) 1356(m) 1232 (w) 1177 (m) 1123 (w) 1059 (w) 1019(w) 944 (m) 866 (w) 813 (w) 742 (s) 700 (w) 616 (w)566(w)

Proton Magnetic Resonance Spectral Data ($CDCl_3$ / TMS), δ in ppm

2.9	s	6H	-N -(CH_3) $_2$
4.6	s	1H	C-H methine

6.7 -7.7 m 12H Aromatic protons
8.1 s 1H C –H benzimidazole

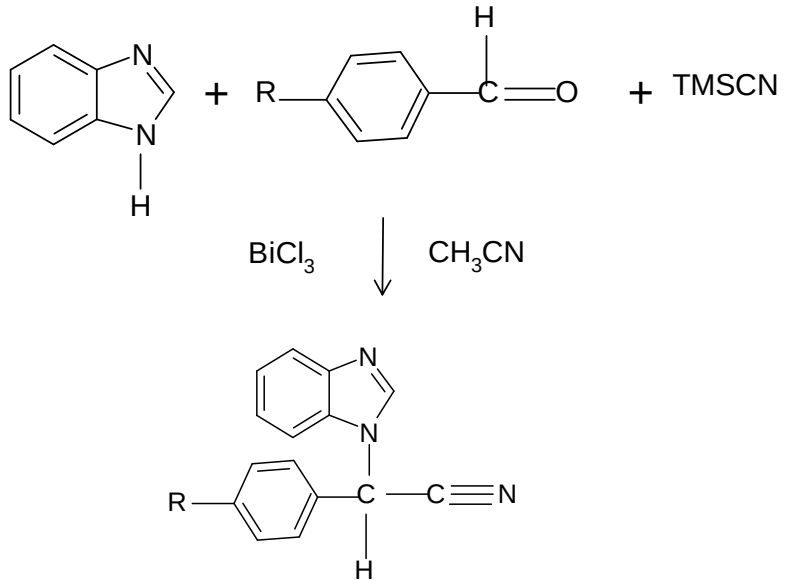
Mass Spectral Values ; m/z and %
368 (30) 367 (50) 324 (48) 278(45) 276 (25) 251 (20) 249(40)248 (30) 247 (12) 207 (15) 205 (20) 161(20) 159(25) 156 (40) 131 (35) 129 (15) 120 (30) 119 (60) 118 (100) 117 (15) 92(35) 91(22) 63 (40)

Elemental Analysis	**C %**	**H%**
C_{23} H $_{20}$ N_4 O Calculated	: 75.00	5.4
M.W . 368 found	: 74.9	5.2

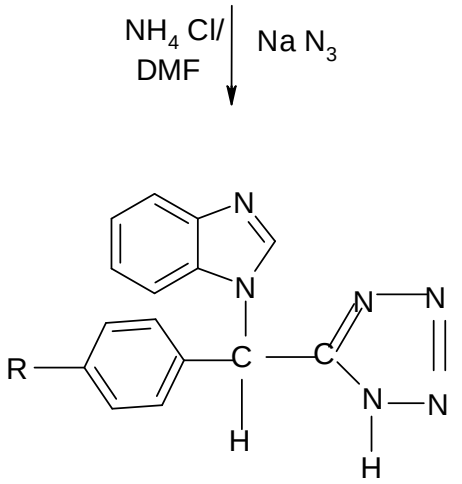
RESULTS AND DISCUSSION
The formation of 2-p-substituted phenyl-2-benzimidazolo acetonitriles (1a-3a) was confirmed by spectral values of IR and NMR and are presented in Table 1. In the present study, 2-(α-p -Substituted phenyl-α-benzimidazolo) methyl -1,2,3,4-tetrazoles were synthesized by condensation of 2 -benzimidazolo -2- phenylacetonitrile(1a) and other nitriles (2a-5a) with sodium azide in the presence of hydrochloric acid . The synthetic route of these compounds were represented as Scheme-I.

Table 1: Spectral data of the compounds (1a-3a)

Sample No	IR (KBr) cm $^{-1}$ (Nitriles)	^{1} H-NMR ($CDCl_3$) δ ppm
1a	2220	4.6(S,1H, -CH methine),6..6-7.4 (m,8H,Ar-H),7.8, (S, 1H,OH phenolic),8.1(s,1H,C-H, Benzimidazole)
2a	2232	3.9(s,3H, - CH_3 anisyl) 4.6(s,1H, -CH methine), 7.0-7.4 (m,8H,Ar-H),8.2(s,1H,C-H, Benzimidazole)
3a	2240	3.0 (s,6H-N -(CH_3) $_2$) 4.6(s,1H, -CH methine), 6.6-7.3(m,8H,Ar-H),8.2(s,1H,C-H, Benzimidazole)



(1a-3a)



(1b-3b)

R = OH, OCH_3 ,-N –$(CH_3)_2$

Scheme. 1: **Synthesis of 2-(α-p -substituted phenyl-α-benzimidazolo) methyl -1,2,3,4-tetrazole (1b-3b)**

Antimicrobial studies

The antibacterial screening of 1b, 2b and 3b compounds inhibited the activity of the following bacteria Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus(Table 2). Thus, compounds **2b**and **3b** showed a higher activity against all the screened bacteria than **1b** . The antifungal screening of 1b, 2b and 3b compounds for the following fungi : Aspergillus niger, Candida albicans and Candida tropicalis(Table 2). All these compounds 1b, 2b and 3b are active against all fungi .

Table 2: Antimcirobial studies of 1b-3b compounds

BACTERIA (ORGANISM)	Zone of inhibition in mm				
	Chloramphenicol	**CONTROL (DMSO)**	**1b**	**2b**	**3b**
Escherichia coli	**27**	**-**	**13**	**13**	**14**
Pseudomonas aeruginosa	**37**	**-**	**14**	**24**	**25**
Staphylococcus aureus	**31**	**-**	**14**	**26**	**28**
Fungus (ORGANISM)	**Ketoconazole**	**CONTROL (DMSO)**	**1b**	**2b**	**3b**
Aspergillus niger	**17**	**-**	**08**	**10**	**9**
Candida albicans	**15**	**-**	**07**	**09**	**9**
Candida tropicalis	**12**	**-**	**08**	**10**	**9**

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