

Synthesis, Characterization and Evaluation of Antioxidant Activity of Novel Combine Heterocyclic.

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ABSTRACT

Benzimidazoles and pyrazole are heterocyclic compounds which have shown potential for application in a variety of pharmacological targets. They are of wide interest because of their diverse biological activity and clinical applications. Biologically active benzimidazole and pyrazole have been known for a long time and they can act as bacteriostats or bactericides, as well as fungicides. This rings system was proved to be very important as it is involved in numerous antiparasitic, antitumor, anti oxidant and antiviral drugs. It is also well known that these molecules are present in a variety of antioxidant and anti allergic agents. Many derivatives of benzimidazole as well as pyrazole show antiparasitic and antiprotozoal activities. As per the literature the by simple nucleophilic reaction we formed the 1-(1H-benzimidazol-1-yl)-2-chloroethanone. Then addition reaction with hydrazine to give 2-(1H-benzimidazol-1-yl) aceto hydrazide. After that we use ethylacetoacetate to give 1-(2-(1H-benzimidazol-1-yl) acetyl)-3-methyl-1H-pyrazol-5(4H)-one by cyclization. Followed by addition of different aldehydes to give newer heterocyclic derivatives. These all newer compounds were characterized by IR, MASS and NMR spectroscopy and evaluate for antioxidant activity.

KEY WORDS

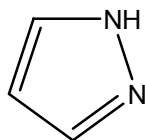
Benzimidazole, Pyrazole, IR, MASS, NMR

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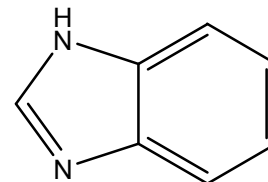
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INTRODUCTION

Drugs are chemicals that prevent disease or assist in restoring health to the diseased individuals as such they play an indispensable role in modern medicine. Heterocyclic compounds having five or six member ring with at least one hetero atom as the ring member, which are relatively stable and exhibit aromatic character. ^[1]



Pyrazole



Benzimidazole

Benzimidazoles and pyrazole are heterocyclic compounds which have shown good for use in a variety of pharmacological targets. They are of wide interest because of their different biological activity and clinical applications. Benzimidazoles and pyrazole have been known for a long time and they can act as antibacterial and antifungal. This rings system was proved to be very important as it is involved in numerous antiparasitic, antitumor, anti-inflammatory as well as antiviral drugs. It is also well known that these molecules are present in a variety of antioxidant and anti allergic agents. Many derivatives of benzimidazole and pyrazole show antiprotozoal activities. ^{[2], [3], [4], [5]}

Benzimidazole:

- Molecular formula: $C_7H_6N_2$
- Molecular weight: 118 gm/mol
- pK_a Value: 12.8

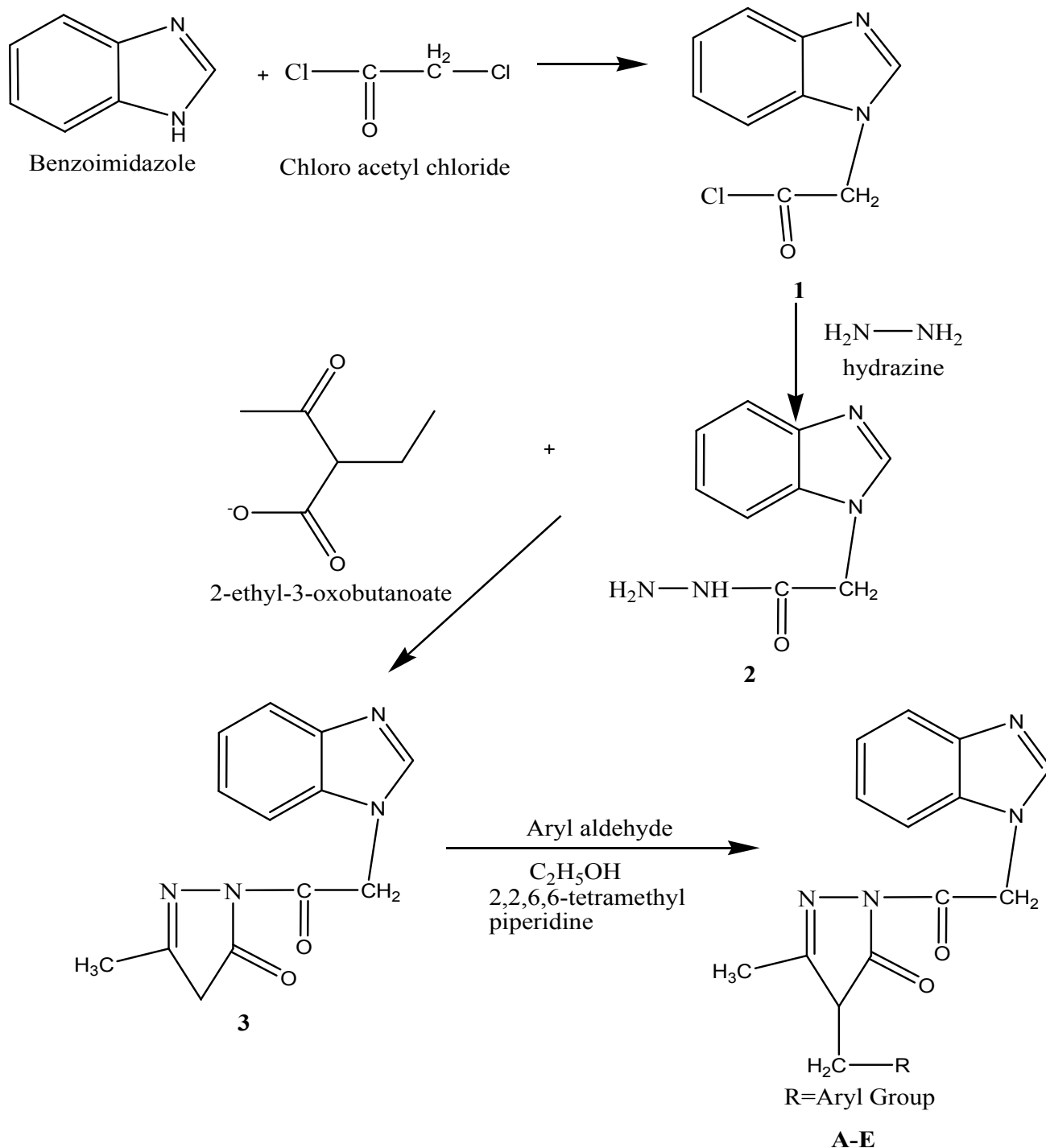
Pyrazole:

- Molecular formula: $C_3H_4N_2$
- Molecular weight: 68 gm/mol

➤ pK_b Value: 11.5

EXPERIMENTAL WORK

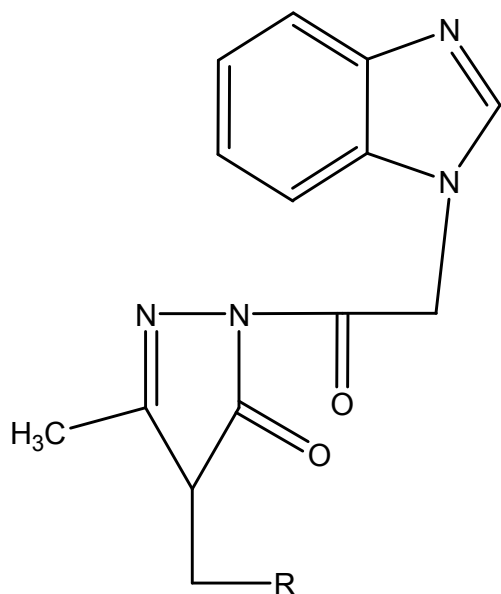
Synthetic scheme ^{[6], [7], [8], [9]}



Spectral characterization

The melting point of the synthesized compounds were determined in open capillary using VEEGO MELTING POINT APPARATUS model VMP-D and recorded in Celsius without correction. The Infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400S spectrometer using KBr as a back ground. Also using JASCO-FTIR 5300 and BUCK SCIENTIFIC INC. M500. NMR spectra of the synthesized compounds were taken using BRUKER ADVANCE-II 400 MHZ, VARIAN MERCURY YH-300 MHZ, and BRUKER ADVANCE-II 400MHZ and GEMINI-200 MHZ “ciba” spectrometer using tetramethyl silane as an internal standard. Mass spectra of the synthesized compounds were taken using 2010EV LCMS SHIMADZU, SHIMADZU GL-MS.

“Table 1: Series of Compounds”



“Table 2: Spectral Information”

Comp. No.	-R
A	
B	
C	
D	
E	

Comp. No.	% Yield	M.P	Mol. Wt.	Mass (m/e)	IR (cm ⁻¹)	¹ HNMR(δ ppm)
A	56.32	190-194°C	364	365 (M+1)	1593 cm ⁻¹ (-C=C-) 3032&2922 cm ⁻¹ (-Ar) 895 cm ⁻¹ (p-substitution) 1467 cm ⁻¹ (-CH ₂) 1676 cm ⁻¹ (-C=O) 1369 & 1327 cm ⁻¹ (-F)	7.1-8.08 (m, 8H, Ar-H) 8.10 (s, 1H, imidazole) 3.5 (d, 2H, -CH ₂) 2.49 (s, 1H, methine, diazole) 3.2, 3.1 (s, 2H, -CH ₂ diazole) 1.04 (s, 3H, -CH ₃ diazole)
B	59.85	143-146°C	362	361 (M-1)	1596&1445 cm ⁻¹ (-C=C-) 3001&2943 cm ⁻¹ (-Ar) 679 cm ⁻¹ (m-substitution) 1456 cm ⁻¹ (-CH ₂) 1711 cm ⁻¹ (-C=O) 3536 cm ⁻¹ (-OH)	7.0-8.0 (m, 8H, Ar-H) 8.1 (s, 1H, imidazole) 4.0 (d, 2H, -CH ₂) 2.4 (s, 1H, methine, diazole) 2.7, 2.8 (s, 2H, -CH ₂ diazole) 0.9 (s, 3H, -CH ₃ diazole) 5.0 (s, 1H, -OH, Aromatic)
C	60.65	133-138°C	362	401.1 (M+K)	1593 cm ⁻¹ (-C=C-) 2922 cm ⁻¹ (-Ar) 950 cm ⁻¹ (o-substitution) 1797 cm ⁻¹ (-CONH) 1676 cm ⁻¹ (-C=O) 3649 cm ⁻¹ (-OH)	7.3-8.0 (m, 8H, Ar-H) 8.1 (s, 1H, imidazole) 3.6 (d, 2H, -CH ₂) 2.4 (s, 1H, methine, diazole) 3.4, 3.3 (s, 2H, -CH ₂ diazole) 1.2 (s, 3H, -CH ₃ diazole) 3.9 (s, 1H, -OH, Aromatic)
D	59.89	155-159°C	362	400.9 (M+K)	1593&1493 cm ⁻¹ (-C=C-) 3032&2922 cm ⁻¹	7.02-8.09 (m, 8H, Ar-H) 8.2 (s, 1H, imidazole) 4.4 (d, 2H, -CH ₂)

					(-Ar) 895 cm^{-1} (p-substitution) 1467 cm^{-1} (-CH ₂) 1676 cm^{-1} (-C=O) 3649 cm^{-1} (-OH)	2.5 (s, 1H, methine, diazole) 3.42, 3.44 (s, 2H, -CH ₂ diazole) 1.1 (s, 3H, -CH ₃ diazole) 4.57 (s, 1H, -OH, Aromatic)
E	55.36	193-199°C	391	391.9 (M+1)	1516&1415 cm^{-1} (-C=C-) 679 cm^{-1} (m-substitution) 1456 cm^{-1} (-CH ₂) 1711 cm^{-1} (-C=O) 1599 cm^{-1} (-CONH) 1371 cm^{-1} (-NO ₂)	6.9-8.1 (m, 8H, Ar-H) 8.2 (s, 1H, imidazole) 4.7 (d, 2H, -CH ₂) 2.4 (s, 1H, methine, diazole) 2.9, 3.0 (s, 2H, -CH ₂ diazole) 0.9 (s, 3H, -CH ₃ diazole) 5.0 (s, 1H, -OH, Aromatic)

EVALUATION OF ANTIOXIDANT ACTIVITY

All the synthesized compounds were evaluated for their antioxidant activity using 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity. Ascorbic acid was used as a standard drug.

Instrument

UV Visible double beam Spectrophotometer (Shimadzu UV 1800)

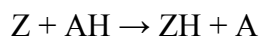
Methods for Anti oxidant activity

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity

This method was given by Brand-Williams, Cuvelier and Berst and later modified by Sanchez-Moreno, Larrauri and Saura-Calixto. It is one of the most extensively used antioxidant assay for plant samples.

This method is based on scavenging of the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) from the antioxidants, which produces a decrease in absorbance at 517 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. This delocalization is also responsible for the deep violet color, characterized by an absorption band in methanol solution at about 517 nm.

Representing the DPPH radical by Z and the donor molecule by AH, the primary reaction is:



Chemicals & reagents

1- 1 diphenyl-2- picryl hydrazyl (DPPH). DPPH reagent is prepared in methanol (1.3 mcg/ml) & protect from light by covering the test tubes with aluminum foil.

Preparation of Test solution

All the drug solutions mentioned above were taken in range of 50 - 200 mcg/ml in methanol.

Preparation of standard solution

Ascorbic acid is used as standard. Aliquot of 10-100 mcg/ml in methanol is prepared.

Procedure

0.1 ml of DPPH (1- 1 diphenyl-2- picryl hydrazyl) solution is added into 3 ml methanol & absorbance is taken after 30 minutes at 516 nm for control reading. 0.1 ml of different concentrations of test compounds and standard were mixed with 0.1 ml of DPPH and diluted up to 3 ml with methanol. Then the mixture is kept in dark for 30 minutes and absorbance is measured at 516 nm after 30 minutes. The absorbance of control reduces dose dependently.

The % reduction was calculated as follows:

$$\% \text{ Scavenging} = (AB - AA / AB) \times 100$$

Where,

AA is the absorbance of the tested sample after 30 minutes.

AB is the absorbance of Control sample.

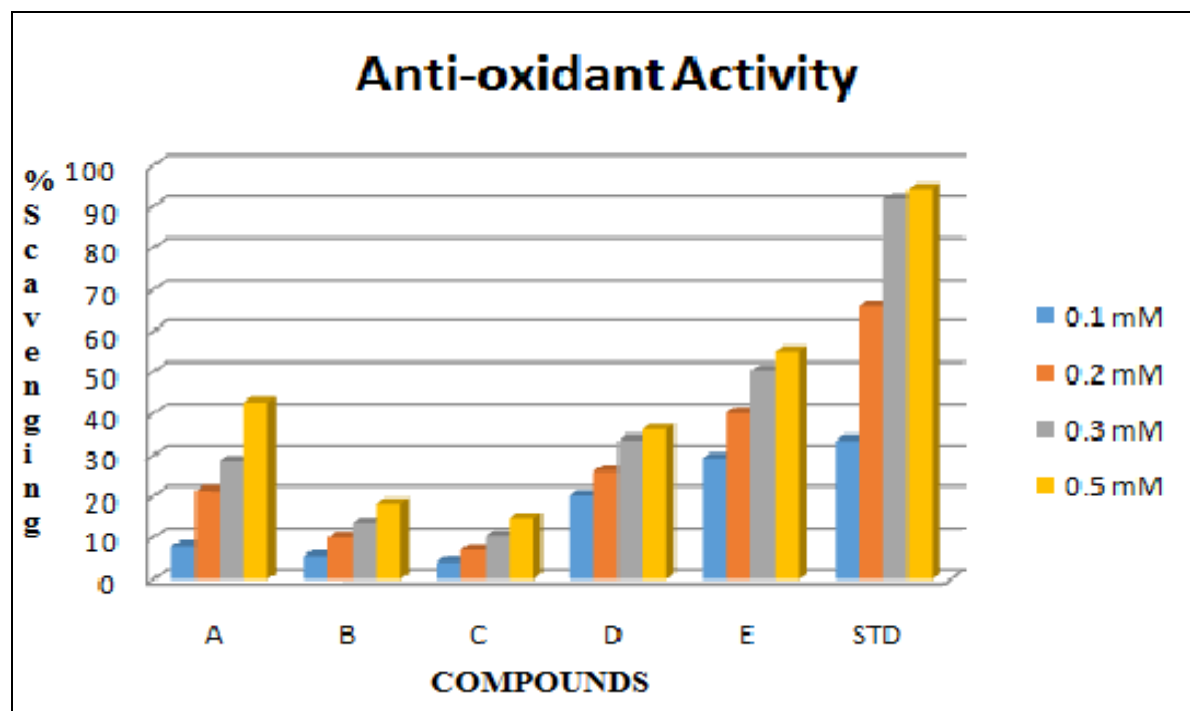
IC50 is the concentration required to reduce % reduction by 50 %.

RESULT AND DISCUSSION

“Table 3: % scavenging data of test compounds and std drug”

Compounds	% scavenging			
	Concentration (mM)			
	0.1	0.2	0.3	0.5
A	7.92	21.25	28.7	42.87
B	5.68	10.16	13.51	18.36
C	4.38	7.18	10.34	14.82
D	20.22	26.09	33.74	36.25
E	29.44	40.24	50.39	55.15
Ascorbic acid	33.64	65.89	91.7	94.32

“Graph-1: Antioxidant activity of test compounds and std drug”



From the table 3 and graph 1, all synthesized compounds showed moderate to good antioxidant activity. The compounds A and E possess good antioxidant activity but less than ascorbic acid. Compound A contain fluorine at p position and compound E contain NO_2 at m position so we can conclude that compound contain fluorine and nitro group are giving good anti oxidant activity. This may be due to both are (F and NO_2) highly electronegative.

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