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

Mr. Ojash Patel*[1], Dr. Mrunal Shirsat [2]

[1] Research scholar, Madhav University, Pindwara, Abu road, Rajasthan, India.

Email-ojas_patel1984@yahoo.com

[2] Dean, Department of Pharmacy, Madhav University, Pindwara, Abu road, Rajasthan, India.

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 nucleophillic reaction we formed the 1-(1H-benzoimidazol-1-yl)-2-chloroethanone. Then addition reaction with hydrazine to give 2-(1H-benzo[d]imidazol-1-yl) aceto hydrazide. After that we use ethylacetoacatate to give 1-(2-(1H-benzo[d]imidazol-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

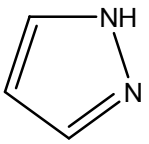
Benzimidazole, Pyrazole, IR, MASS, NMR

*Corresponding author

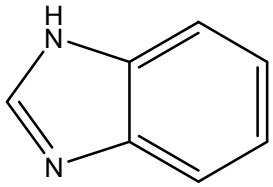
Mr. Ojash Pate, Research scholar, Madhav University, Pindwara, Abu road, Rajasthan, India. Mob-09825808094, Email- ojas_patel1984@yahoo.com.

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

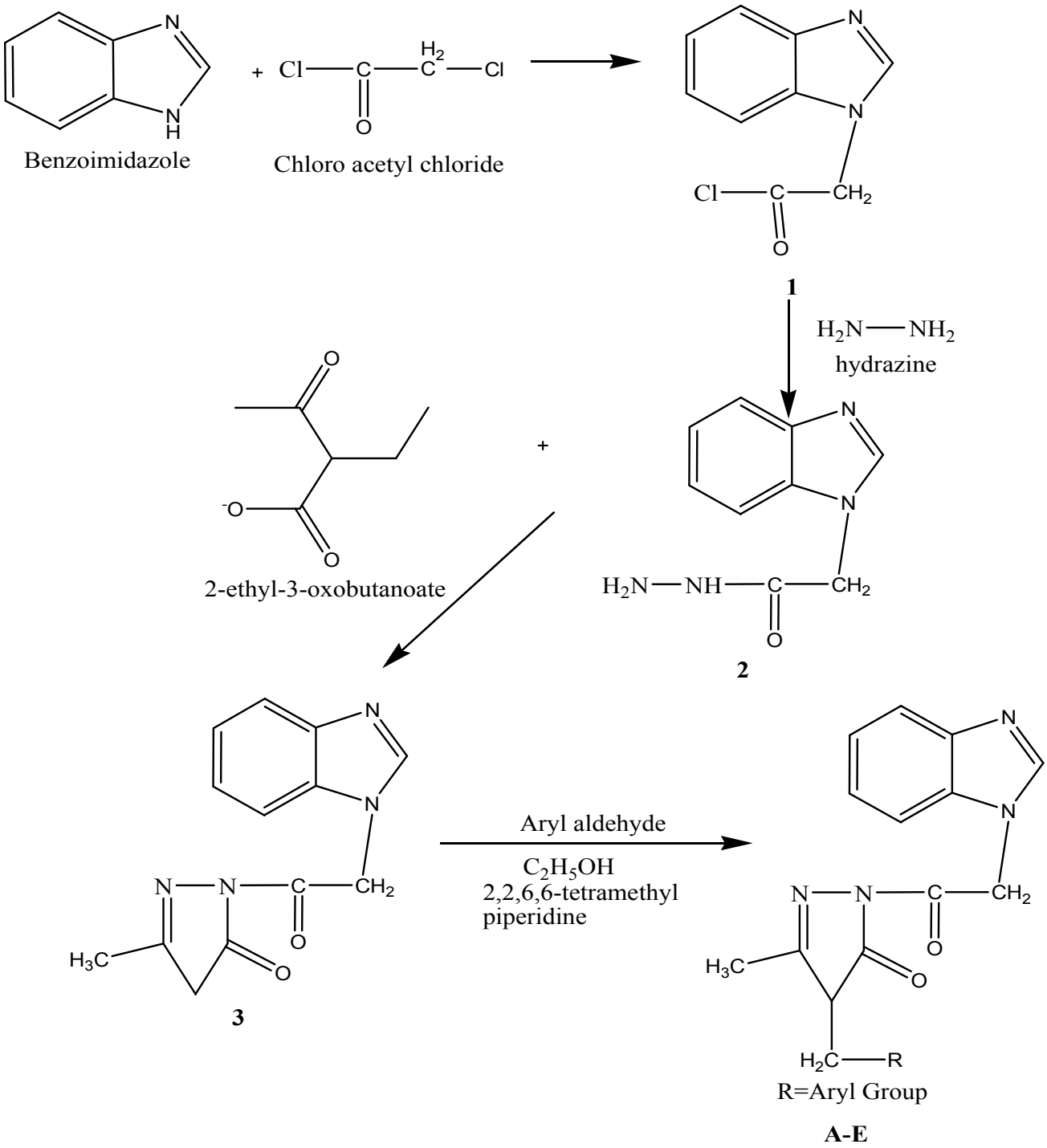
Pyrazole:

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

- pK_b Value: 11.5

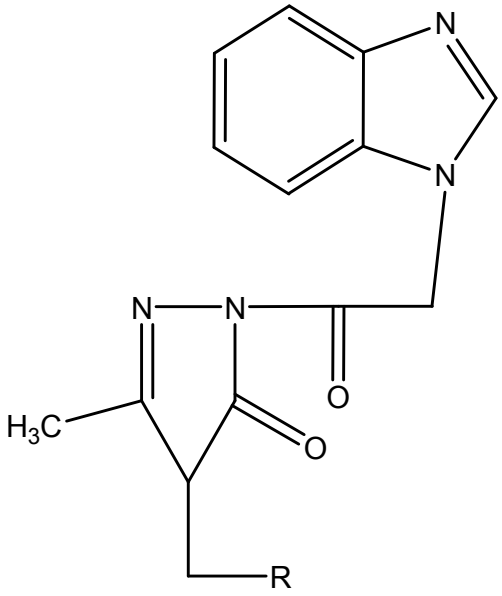
EXPERIMENTAL WORK

Synthetic scheme [6], [7], [8], [9]



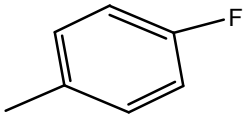
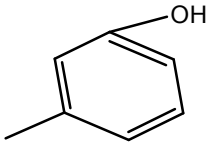
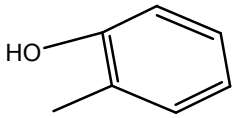
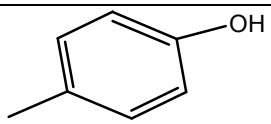
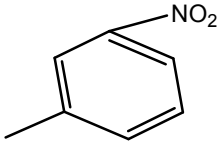
Spectral characterization

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-3OO 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 2: Spectral Information"

"Table 1: Series of Compounds"

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, -CH2) 2.49 (s, 1H, methine, diazole) 3.2, 3.1 (s, 2H, -CH2 diazole) 1.04 (s, 3H, -CH3 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, -CH2) 2.4 (s, 1H, methine, diazole) 2.7, 2.8 (s, 2H, -CH2 diazole) 0.9 (s, 3H, -CH3 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, -CH2) 2.4 (s, 1H, methine, diazole) 3.4, 3.3 (s, 2H, -CH2 diazole) 1.2 (s, 3H, -CH3 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, -CH2)

					(-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, -CH2 diazole) 1.1 (s, 3H, -CH3 diazole) 4.57 (s, 1H, -OH, Aromatic)
E	55.36	193-199˚C	391	391.9 (M+1)	1516&1415cm^{-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, -CH2) 2.4 (s, 1H, methine, diazole) 2.9, 3.0 (s, 2H, -CH2 diazole) 0.9 (s, 3H, -CH3 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 decreases in absorbance at 517 nm. When a solution of DPPH is mixed with a substances that can donate a hydrogen atom, the reduced from 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:

$$Z + AH \rightarrow ZH + A$$

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 add 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 dilute it up to 3 ml with methanol. Than the mixture is kept in dark for 30 minutes and absorbance is measured at 516 nm after 30 minutes. The absorbance of control reduce dose dependently.

The % reduction was calculated as follow:

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

Where,

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

AB is the absorbance of Control sample.

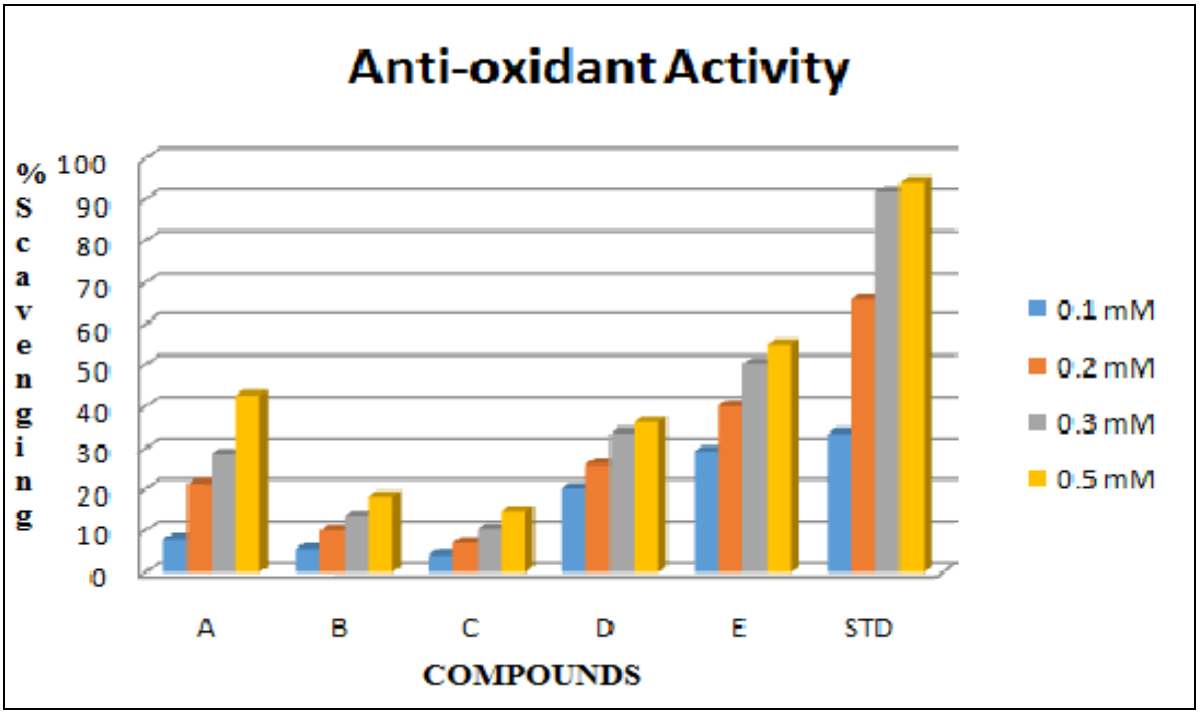
IC50 is the concentration required to reduce % reduction by 50 %.

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: Antioxident 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.

ACKNOWLEDGEMENT

We would like to special thanks the Principal and Management of Faculty of pharmacy, Shree Satsangi Saketdham "Ram Ashram" group of institutions, Vadasma, Mahesana, Gujarat for providing laboratory facilities.

REFERENCES

1. Patel OB, Patel LJ, Microwave assisted synthesis and biological evaluation of benzimidazole derivatives as anticancer agents, International journal of pharmaceutical and applied sciences, 2011; 2(1): 16-19.
2. Zhang SS, Zhang HO, Sun LH, Ma CP, Wang W, Wan J, et al. A novel benzotriazole derivative inhibits proliferation of human hepatocarcinoma cells by increasing oxidative stress concomitant mitochondrial damage, European Journal of Pharmacology, 2008; 584: 144–152.
3. Desai KG, Desai KR, Green route for the heterocyclization of 2-mercaptobenzimidazole into β-lactum segment derivatives containing –CONH– bridge with benzimidazole: Screening in vitro antimicrobial activity with various microorganisms, Bioorganic & Medicinal Chemistry, 2006; 14: 8271–8279.
4. Ansari KF, Lal C, synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents, European Journal of Medicinal Chemistry, 2008; 1-6.
5. Lukas J, Goossen, Thomas K, Synthesis from 4-amino-3-methylbenzoic acid methyl ester, J. Org. Chem., 2008; 73(21): 8631-8634.
6. Kittur BS, Sastay BS, Pattan SR, Rabara P, Synthesis and anti-inflammatory activity of some novel 1,3,4-oxadiazole and pyrazoles derivatives, Indian drug, 2009; 46(4): 287-290.
7. Kamila S, Ankati H, Biehl ER, Microwave assisted synthesis of novel functionalized hydantoin derivatives and their conversion to 5-(Z) arylidene-4H-imidazoles, Molecules, 2011; 16: 5527-5537.
8. Galal SA, Khairat SHM, Ali HI, Shouman SA, Attia YM, Ali MM et al. New candidates of pyrazole-benzimidazole conjugates as checkpoint kinase 2 (Chk2) inhibitors, Eur J Med Chem., 2018; 144: 859-873.
9. Patel Ojas, Prajapati Paresh, Synthesis of newer heterocyclic molecules using combination of benzimidazole and pyrazole, Journal of Pharmaceutical and Scientific Innovation, 2013; 2(1): 25-27.
10. Indian Pharmacopoea, Govt. of India: New Delhi, App. 9, A-100 to A-124, 1996.
11. National committee for clinical laboratory standards, 1998.
12. Geeta Mehta, V.S.Ranghawala, K.B.Sharma, practical & viva in medicinal microbiology, 344.