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Research Article

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SYNTHESIS AND BIOLOGICAL SCREENING OF SOME SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Synthesis of commercially available benzimidazole involves condensation of o-phenylenediamine with formic acid. The most prominent Benzimidazole compound in nature is N-riosyl dimethyl benzimidazole. Benzimidazoles are an important class of compounds with a wide spectrum of biological activity like anti-hypertensive, anti-viral, anti-inflammatory, anti-oxidant, anti-fungal, antitumor and anthelmintic. The five membered heterocyclic moiety with substituted amines, aniline, amides also confers for various biological activity. Hence a series of benzimidazole ethanone derivatives fused with phenylhydrazone ring system have been synthesized, characterized by UV,IR and 1HNMR spectral data and evaluated for their in vitro and in vivo anti-inflammatory and antioxidant activity. The substituted benzimdazoles are summarized in this review to know about the chemistry as well as Pharmacological activity.@2012 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND licence (http://creativecommons.org/licenses/by-nc-nd/3.0/).

KEYWORDS: Benzimidazoles, antioxidant, anti-inflammatory activity and pharmacological activity.

1. INTRODUCTION

Imidazole is the accepted name for the parent compound in the series, numbering of which follows the accepted pattern for heterocyclic compound.Imidazole or iminazoline is an azapyrole, the nitrogen atom is separated by one carbon atom. This compound was earlier also called as glyoxalin as it was first prepared in 1958 from glyoxal and ammonia. Benzimidazoles are an important class of compounds with a wide spectrum of biological activity ranging from anti-hypertensive1, anti-viral2, anti-fungal3, antitumor4 and anthelmintic activity5. In addition, few N-substituted benzimidazole derivatives have shown to exhibit significant activity against several viruses, including HIV, herpes simplex (HSV-1), influenza, picorna, human cytomegalovirus (HCMV) and hepatitis C virus. Furthermore, substituted benzimidazoles are potent inhibitors of the parietal cell proton pump, the H+/K+ ATPase, and also are capable of blocking gastric acid secretion in response to known stimuli. 1,3,4-Oxadiazoles are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities. Molecules containing a 1,3,4-oxadiazole core have been shown to have a broad range of important biological activities including antibacterial,[6] antimicrobial,[7,8] pesticidal,[9] anti-mycobacterial,[10] anti- inflammatory[11,12] anti-fungal,[13] anti-cancer,[14] and antihypertensive properties. The widespread use of diazoles as a scaffold in medicinal chemistry establishes this moiety as an important bioactive class of heterocycles. These molecules are also utilized as pharmacophores due to their favorable metabolic profile and ability to engage in hydrogen bonding. Hence an attempt has been made to synthesize some novel compounds of benzimidazoles containing five membered diazole moiety and evaluate for their in vitro and in vivo anti-inflammatory and antioxidant activity.

1.2 MATERIALS AND METHODS

Melting points were measured in open capillary tubes. Melting points were determined using VEEGO Digital apparatus and are uncorrected. IR (KBR) spectra were recorded in film or in potassium bromide disks on a Perkin- Elmer spectrophotometer (υ 4000 -400cm-1) and 1H NMR spectra on a BRUKER Advanced II 400 MHz NMR spectrophotometer. The chemical shifts were reported as parts per million (δ 0-8 ppm) tetra methyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL GC instrument using fast atom

bombardment (FAB positive). The progress of the reaction was monitored on a readymade silica gel plates (Merck) using Chloroform: Methanol (3:7) as a solvent system. Spectral data (IR, and 1HNMR spectra) confirmed the structure of the synthesized compounds and the purity of these compounds were ascertained by microanalysis.

1.3 Procedure

STEP-1: synthesis of 2-[1-(2-phenyl hydrazono) ethyl]1h Benzimidazole.

- A mixture of benzimidazole thanone 3.2 gms and phenyl hydrazine (0.1mole), 50 ml of acetic acid and methanol (25ml) was refluxed for 3 hours
- At the end of this period the mixture was cooled and poured into ice cold water.
- The seperated solid was filtered,washed with water and dried to get crude product which on recrystallized from hot methanol gives pure 2-[1-(2-phenyl hydrazono)ethyl] 1H benzimidazole (compound-1).

Step-2: Synthesis of 2-(1H Indole-2YL)1-H Benzimidazole.

- A mixture of poly phosphoric acid (PPA) 25 ml and 2-[1-(2-phenyl hydrazono)ethyl]1H benzimidazole (compound-1) 0.1 mole in a 100ml round bottomed flask was heated with occasional stirring at 80˚C for 4 hours.
- At the end of this period, the mixture was cooled and poured into ice cold water.
- The separated solid was filtered.
- The filtered solid was treated with a few drops of ammonia solution.
- The resulting solid was filtered and dried to obtain 2-(1H indol-2yl)1-H benzimidazole (compound-2).
- The crude product obtained above was re crystallized from methanol-DMF solution to obtain pure compound-2.

Step 3: Synthesis OF 2-(1H Indol-2YL)1-Aryl-H Benzimidazole.

- A mixture of compound-2 (0.1 mole), K2CO3 (Pottasium permanganate) 0.1 mole, benzyl triethyl ammonium chloride (TEBAC) 10mg, acetonitrile (CH3CN) 20ml, and alkylating agent (0.1 mole) in a round bottomed flask was heated with occasional stirring for 5 hours.
- At the end of this period the mixture was poured into ice cold water.
- The seperated solid was filtered and dried to obtain 2-(1H-indol-2yl)-1-aryl-1H-benzoimidazole (compound-3), which are recrystalized from hot methanol to obtain compound-3.

Table 1: Physical data of synthesized compounds.

Compound code	Molecular formula	Mol.wt	m.p 0 C	% yield	Rf *
C1	C15H14N4	250	96ºC	73%	0.06
C2	C15H11N3	233	105ºC	76%	0.41
S1	C22H18N4	338.15	86ºC	71%	0.6
S2	C22H17N3	323.14	99ºC	75%	0.96
S3	C22H16N4O	352.13	107-115ºC	78%	0.82
S4	C27H20N4	400.17	102ºC	79%	0.67
S5	C17H15N3	261.13	103.37ºC	77%	0.74
S6	C17H14N4O	290.12	79-81ºC	74%	0.8

TLC Solvent- Chloroform**:** Methanol (3:7)

Table 2: Spectral data of the Synthesized derived compounds.

Compound code	Compound IUPAC Name	IR(KBr)Cm-1	1HNMR(CDCl3,)
C1	2-[1-(2-PHENYL HYDRAZONO)ETHYL]1H BENZIMIDAZOLE	C=N str-1697.45, C=C str-1541.36, C-H str-3107.26.	————
C2	2-(1H Indole-2YL)1-H Benzimidazole	C=o str-1768.56, C-N str-1698.39, C=C str-1579.31, N-H bend-1337.94.	————
S1	N-benzyl-2-(1H-indol-2-yl)-1-H-benzo[d]imidazol-1-amine	————	7.02-7.49 delta - m,6H,CH, 6.76-6.86 delta- d,2H,CH, 5.35 delta ------ d,2H,CH2, 4.0 delta----------d,2H,NH.
S2	N-benzyl-2-(1H-indol-2-yl)-1-H-benzo[d]imidazole	————	7.02-7.49 delta - m,4H,CH, 6.76-6.86 delta- d,6H,CH, 5.35 delta ------ d,2H,CH2, 4.0 delta----------d,2H,NH.

***In-Vitro* Anti-Inflammatory Activity**

Inflammation is normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents and local response of living mammalian tissue to injurious agents, which may be due to physical agents like heat, cold, radiation, trauma; Chemical agents like antigen-antibody reactions, call mediated reaction. In the present study invitro anti-inflammatory activity was checked for the synthesized compounds.

1.4 HRBC Membrane Stabilisation method

The method involves the stabilization of human red blood cell membrane by hypo tonicity induced membranelysis.

Principle

The lysosomal enzymes released during inflammatory condition produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membrane since the human red blood cell membrane are similar to lysosomal membrane components. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug.

Reagents

- HRBC suspension : 10 %
- Alsiever solution
- Isotonic saline : 0.85 %
- Phosphate buffer : 0.15M,pH-7.2
- Hypotonic saline : 0.36 %

Preparation of Alsievier's solution: 2g dextrose + 0.8g sodium citrate + 0.05g citricacid + 0.42g sodium chloride was made up with distilled water to 100ml.

Preparation of 0.5 ml of 10 % HRBC Suspention

To 3 ml of blood, add 3 ml of Alsievier's solution and centrifuge at 3000 rpm for 20 minutes then packed calls were washed with isotonic saline and later 10% v/v suspention of the packed cells was made with isotonic saline.

Preparation of Hypotonic Saline

0.36g of sodium chloride in 100 ml of distilled water.

Preparation of isotonic saline

0.85g of sodium chloride in 100 ml of distilled water.

Procedure

The synthesized compounds are to be used for this study. They are to be made into doses of 1000µg/ml with DMSO (5.0 %) solution. Diclofenac sodium is taken as standard. The reaction mixture (4.5 ml) consist of 2 ml of hypotonic saline (0.36 % sodium chloride). 1 ml of o.15 M phosphate buffer (pH 7.4), 1 ml of the test solution (1000 µg/ml) in normal saline and 0.5 ml of HRBC suspention in normal saline. For control test, 1 ml isotonic solution is to be used instead of test solution while product control lacked RBC. The mixture is then incubated at 56°c for 30 minutes, then to be cooled under running tap water and centrifuged at 3000rpm for 20 minutes. The absorbances of the supernatants are read at 560 nm. Percent membrane stabilization activity is calculated as follows:

$$\% \text{ Stabilization} = \frac{\text{OD of test control} - \text{OD of test sample}}{\text{OD of test control}} * 100$$

***IN-Vitro* Antioxidant Activity**

An antioxidant is a molecule capable of slowing or preventing the oxidation or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reaction by being oxidized themselves. Hydrogen peroxide scavenging activity is one of the methods for determining antioxidant *in-vitro* activity.

1.5 Nitric Oxide Radical-Scavenging Activity

Reagents

- Sodium nitroprusside
- Standard phosphate buffer solution
- Griess Reagent (mixing the equal volume of 1% sulphanilamide in 2% phosphoric acid & 0.1%naphthly ethylene diaminedihydrochloride in water.

Standard: Ascorbic acid.

Preparation of 10Mm Sodium nitroprisside

2.979gm in 100ml water, to this pipette out the 10ml and made upto 100ml.

Preparation of 0.1% NEDD

0.1 gm of NEDD (i.e. 100gm) and made upto 100ml of distilled water.

Preparation of test solution

In the assay, 2ml of sodium nitroprusside **(10Mm)** in 0.5ml phosphate –buffered saline (PBS) was mixed with 0.5ml of different concentration of sample ranging from (50-250µg/ml) prepared in methanol and incubated at 25°c for 150min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30min, 1.5ml of incubated solution was removed and diluted with 1.5ml of Griess reagent. Absorbance of chromosphore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with NEDD was measured at 546nm and the percentage scavenging activity measured with reference to the standard.

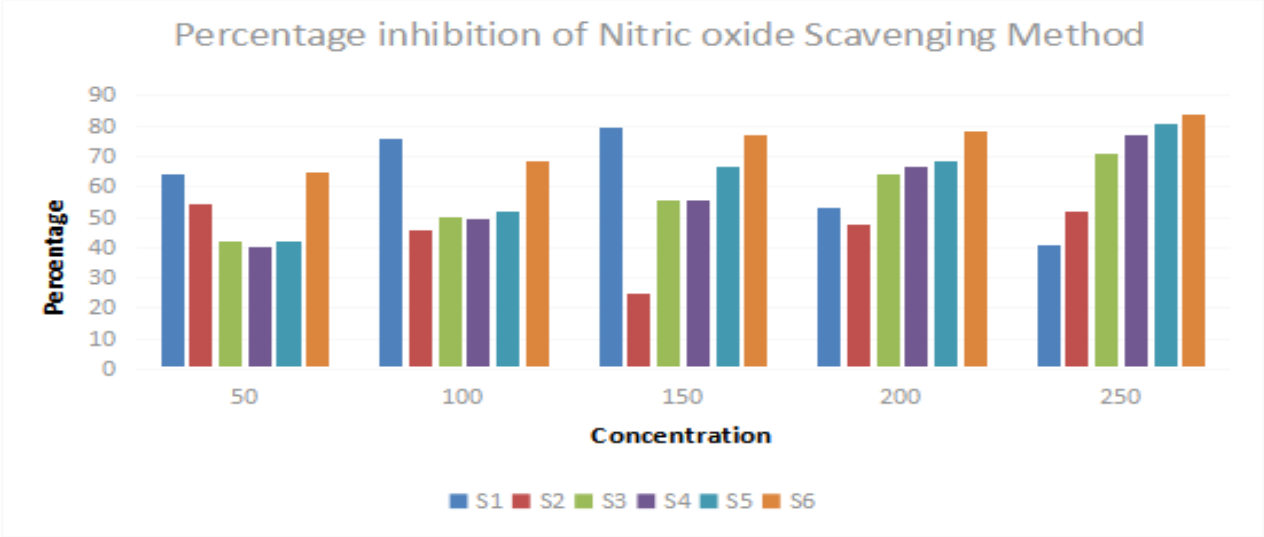
Abs.control-Abs.of test

$$\% \text{ inhibition} = \frac{\text{Abs. control}}{\text{Abs. control}} \times 100$$

Nitric oxide Scavenging Effect (% inhibition) of BenzimidazoleDerivaties

Compound Code	50µg/ml	100µg/ml	150µg/ml	200µg/ml	250µg/ml
S1	64.4	76	79.6	53.2	40.8
S2	54.2	45.9	24.9	47.8	51.8
S3	42.1	50.2	55.8	64.2	71.2
S4	40	49.7	55.8	66.7	77.1
S5	42.1	51.8	66.4	68.6	80.6
S6	48.6	58.6	65.4	71.7	83.0
STD	64.6	68.6	76.9	78.1	83.7

Percentage Inhibition of Nitric oxide Scavenging Method



RESULTS AND DISCUSSION

***In-vitro* Anti-inflamatory activity**

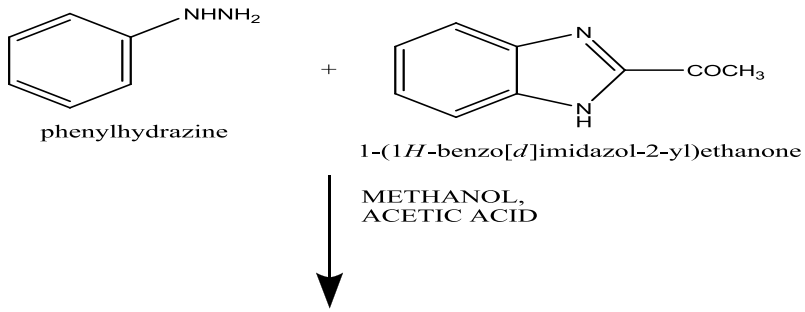
The synthesized compounds were subjected to *in-vitro* anti-inflammatory activity using HRBC membrane stabilizing method. The method involves the stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the drug. The compound A3 showed better activity as compared to the standard diclofenac. Rest of the compounds showed moderate activity.

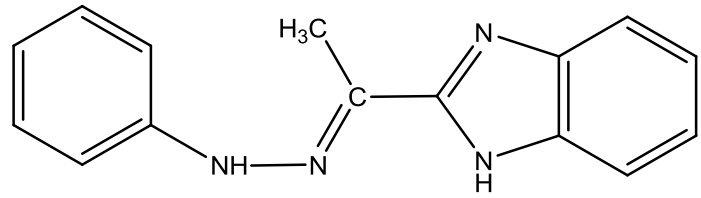
Anti oxidant activity revealed that all the synthesized compounds have showed significant antioxidant activity when compared with that of standard by Hydrogen peroxide and Nitric oxide scavenging methods.

Anti inflammatory activity revealed that all the synthesized compounds have showed significant activity when compared with that of standard by HRBC membrane stabilization method.

S2 compound will shows more antioxidant activity than compare to the other and S3 compound shows more Anti-inflammatory compare to other standard diclofenac sodium.

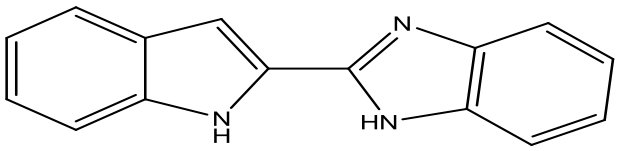
Scheme





2-(1-(2-phenylhydrazono)ethyl)-1*H*-benzo[*d*]imidazole

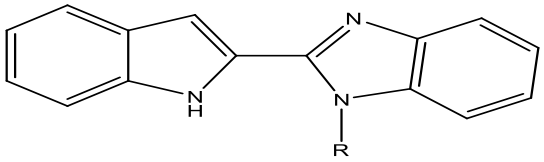
POLY PHOSPHORIC ACID,
140·C, 3 Hrs Reflux.



2-(1*H*-indol-2-yl)-1*H*-benzo[*d*]imidazole

Various aromatic amines, amides and aldehydes.

Benzyl tri ethyl ammonium chloride,
Potassium Carbonate,
Dimethyl formamide,
Acetonitrile.



2-(1H indol-2yl)-aryl)1H-benzo imidazole

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