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Synthesis, Anti-Inflammatory and Anti-oxidant activity of some substituted Benzimidazole Derivatives

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Abstract

Benzimidazoles are an important class of compounds with a wide spectrum of biological activity ranging from anti-hypertensive, anti-viral, anti-fungal, antitumor and anthelmintic activity. 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. The five membered heterocyclic moiety 1,3,4-oxadiazole also confers for various biological activity. Hence a series of benzimidazole derivatives fused with oxadiazole ring system have been synthesized, characterized by UV, IR and ^{1}H NMR spectral data and evaluated for their *in vitro* and *in vivo* anti-inflammatory and antioxidant activity.

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Introduction

Benzimidazoles are an important class of compounds with a wide spectrum of biological activity ranging from anti-hypertensive[1], anti-viral[2], anti-fungal[3], antitumor[4] and anthelmintic activity[5]. 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 1,3,4 -oxadiazoles 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 1,3,4-oxadiazole moiety and evaluate for their *in vitro* and *in vivo* anti-inflammatory and antioxidant activity.

MATERIALS AND METHODS

Melting points were measured in open capillary tubes and are uncorrected. IR (KBR) spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 39 spectrophotometer (υ max in cm-1) and 1H NMR spectra on a DPX 300 MHz Bruker FT-NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm) tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on a readymade silica gel plates (Merck) using n-hexane: ethyl acetate as a solvent system. Spectral data (IR, 1HNMR and Mass spectra) confirmed the structure of the synthesized compounds and the purity of these compounds were ascertained by microanalysis.

Procedure:

Synthesis of (E)-(1*H*-benzo[*d*]imidazole-1-yl) (4-(2-hydroxybenzylideneamino) phenyl) methanone (1a):

Benzimidazole (0.03m) and (*E*)-ethyl 4-(2-hydroxy benzylideneamino)benzoate (0.03m) were taken in a dry round bottom flask containing 25 ml of dry pyridine and refluxed for 6-7h. The reaction mixture was cooled and poured into crushed ice to obtain the solid. Recrystallized from alcohol.

Synthesis of (*E*)-(1*H*-benzo[*d*]imidazole-1-yl)(4-(4-chlorobenzylideneamino) phenyl) methanone (1b):

Benzimidazole (0.03m) and (E)-ethyl 4-(4-chlorobenzylideneamino)benzoate (0.03m) were taken in a round bottom flask containing dry pyridine and refluxed for 18 h. The reaction mixture was cooled and poured into crushed ice to obtain the solid.

Synthesis of 5-Phenyl -1,3,4-oxadiazol-2-amine (2):

Benzohydrazide (0.02m) and cyanogen bromide (0.02m) were taken in ethanol in a round bottom flask. The reaction mixture was heated on a water bath at 50-60°C for 30 min. It was cooled and neutralized with sodium bicarbonate. The reaction mixture was poured into beaker containing crushed ice. A solid was formed which was filtered, washed thoroughly with water, dried and recrystallised from alcohol.

Synthesis of 2-chloro-*N*-(5-phenyl-1,3,4-oxadiazole-2-yl)acetamide (3):

2-Amino-5-phenyl-1,3,4-oxadiazle (0.013m) was transferred to a round bottom flask containing distilled and dried benzene. Chloroacetyl chloride (0.018m) was added dropwise for 15min with constant stirring at cold condition. After the complete addition of chloroacetyl chloride the reaction mixture was stirred for 3h at room temperature and warmed on a water bath for 30 min. The excess benzene was distilled off and the solid obtained was collected.

Synthesis of 2-(1*H*-benzo[*d*]imidazole-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (1c):

Benzimidazole (0.04m) and 2-chloroacetamido-5-phenyl-1,3,4-oxadiazole (0.04m) were taken in a round bottom flask containing dry pyridine and refluxed for 5h. The content was cooled and extracted with chloroform to obtain the solid substance.

Synthesis of 1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone (4):

Benzimidazole (0.016m) was treated with chloroacetyl chloride (0.34m) dropwise for 15 mins with constant stirring at cold condition. After the complete addition of chloroacetyl chloride the reaction mixture was stirred for 3h at room temperature and warmed on a water bath for 30m. The excess benzene was distilled off and the solid obtained was collected.

Synthesis of 5-Phenyl-1,3,4-oxadiazole-2-thiol (5):

Benzohydrazide (0.036m) and carbon disulfide (0.08m) were taken in round bottom flask, potassium hydroxide solution (0.03m) was added to it. The reaction mixture was refluxed until the evolution of hydrogen sulfide ceased, then cooled, diluted with cold water and acidified with glacial acetic acid. The solid separated was washed with water and recrystallized from alcohol.

The compounds 5-(pyridin-4-yl) -1,3,4-oxadiazole-2-thiol **(6)** and 2-(5-mercapto-1,3,4-oxadiazol-2-yl)phenol **(7)** were synthesized following the same procedure using isonicotinic acid hydrazide and 2-hydroxy benzohydrazide recspectively.

Synthesis of 1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-phenyl-1,3,4-oxadiazole-2-ylthio)ethanone (1d):

1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone (0.007m) was taken in round bottom flask containing dry pyridine. 5-Phenyl-1,3,4-oxadiazole-2-thiol (0.007m) was added and the reaction mixture was refluxed for 28 h and poured into a beaker containing crushed ice and extracted with chloroform to obtain the solid.

The compounds 1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)ethanone **(1e)** and 1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2-ylthio)ethanone **(1f)** were synthesized following the same procedure. The physical and spectral data of the synthesized compounds are given in Table 1&2.

Table 1: Physical data of the synthesized compounds

Comp Code	Mol formula	Mol.wt	m.p °C	% yield	R_f*
1a	$C_{21}H_{15}N_3O_2$	341	90	39.50	0.71
1b	$C_{21}H_{14}N_3OCl$	359	156	35.29	0.54
1c	$C_{17}H_{13}N_5O$	303	110	17.00	0.26
1d	$C_{12}H_{12}N_4SO_2$	336	177	39.76	0.91
1e	$C_{16}H_{10}N_5SO_2$	336	187	30.01	0.48
1f	$C_{17}H_{12}N_4SO_3$	352	154	45.22	0.53

* TLC Solvent - *n* Hexane: Ethyl acetate = 1:3

Table 2: Spectral Data of the synthesized compounds

Comp Code	Compound	UV (λmax)	IR (KBr)cm^{-1}/ ^{1}H NMR ($CDCl_3$, δ)
1a	(E)-(1*H*-benzo[*d*]imidazole-1-yl)(4-(2-hydroxyenzylidene amino) phenyl) methanone	343	3396 OH str, 3064 ArCH str, 2974 Ali CH str, 1705 CO str, 1596 CN str,
1b	(*E*)-(1*H*-benzo[*d*]imidazole-1-yl)(4-(4-chloro benzylidene amino) phenyl) methanone	297	3062 ArCH str, 2970 Ali CH str, 1710 CO str, 1598 CN str,
1c	2-(1*H*-benzo[*d*]imidazole-1-yl)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide	299	3341 NH str, 3062 ArCH str, 1647 CO str, 1509 CN str
1d	1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-phenyl-1,3,4-oxadiazole-2-ylthio)ethanone	273	3065 ArCH str, 2856 Ali CH str, 1586 CO str, 1457 CN str
1e	1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)ethanone	318	3064 Ar CH str, 2835 Ali CH str, 1595 CO str, 1462 CN str. 8.87 – 6.74 (m, 8H, Ar H), 6.64 (s, 1H, Ar H Benzimidazole), 2.18 (s, 2H, COCH2),
1f	1-(1*H*-benzo[*d*]imidazole-1-yl)-2-(5-(2-hydroxyphenyl)-1,3,4-oxadiazole-2-ylthio) ethanone	--	3442 OH str, 3066 Ar CH str, 2883 Ali CH str, 1595 CO str, 1409 CN str

***Invitro* anti-inflammatory activity[15]:**

A solution of 0.2% w/v of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10000 µg/mL of all test samples were prepared by using methanol as a solvent. From these stock solutions two different concentrations 100 µg/mL and 200 µg/mL were prepared by using methanol as a solvent. 100µl of

each test sample was transferred to 0.1mL Eppendrof tubes using 1mL micropipette. 5mL of 0.2% BSA was added to all the above tubes. The control consists of 5mL 0.2%w/v BSA solution with 50µl methanol. The 0.1mL standard consist 100µg/mL of Diclofenac sodium in methanol with 5mL 0.2%w/v BSA solution. The test tubes were heated at 720C for five minutes and then cooled for 10 min. The absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control.

Table 3: *Invitro* Antiinflammatory activity

Sl. No	Comp Code	% inhibition	
		100µg/ml	200 µg/ml
1	1a	37.26	43.47
2	1b	5.59	31.67
3	1c	5.59	18.63
4	1d	37.88	63.35
5	1e	42.23	63.35
6	1f	36.78	58.20
	Diclofenac	66.45	68.94

Invivo anti-inflammatory activity[16]:

The initial paw volume of each rat was noted by mercury displacement method using plethysmograph. Animals in the group-1 was administered with 2.5%DMSO+2.5% tween 20, the group-2 received indomethacin at a dose of 10 mg/kg body weight, where as group 3-10 received the test samples. After the drug treatment, 1% w/v Carrageenan solution (0.1 ml/paw) was injected subcutaneously into the plantar surface of the right hind paw of the rat. The paw volume of the legs of control, standard & tested groups was measured with the help of plethysmograph during the time interval of 30th, 60th, 120th, 180th and 240th min after carrageenan administration.

Percentage protection (or inhibition) was calculated by using the formula,

% protection = (1- Vt / Vc) X 100, where

Vt is the mean increase in the paw volume in the test animals group, Vc is the mean increase in the paw volume in the control group (in anti-inflammatory study).

Table 4: *Invitro* Anti-inflammatory activity

	Dose	mean paw volume in ml ±SEM				
		30min	60min	120min	180min	240min
Control		0.329±0.004	0.368±0.008	0.520±0.006	0.659±0.006	0.747±0.007
Indomethacin	10mg/kg	0.259±0.007[a]	0.319±0.004[a]	0.361±0.008[a]	0.347±0.009[a]	0.384±0.005[a]
1a	10mg/kg	0.307±0.007[a,b]	0.355±0.008[a,b]	0.407±0.008[a,b]	0.560±0.005[a,b]	0.577±0.003[a,b]
1b	10mg/kg	0.390±0.008[a,b]	0.399±0.008[a,b]	0.420±0.007[a,b]	0.499±0.007[a,b]	0.546±0.005[a,b]
1c	10mg/kg	0.356±0.008[a,b]	0.323±0.004[a,b]	0.447±0.009[a,b]	0.478±0.006[a,b]	0.582±0.007[a,b]
1e	10mg/kg	0.388±0.010[a]	0.323±0.007[a]	0.374±0.005[a]	0.375±0.007[a]	0.423±0.005[a]

All the data are expressed as mean ± SEM , a=p<0.001 when compared to control, b= p<0.001 when compared to standard.

Antioxidant Activity:

Evaluation of the antioxidant potential of all the compounds *in-vitro* free radical scavenging activity using DPPH (2,2-diphenyl-1-picryl hydrazyl) reduction method.

10 mg of DPPH was dissolved in 10 ml of methanol. From this stock solution dilutions were made to obtain concentrations of 10, 20, 30, 40 µg/ mL. The absorbance was recorded for these dilutions at 516 ηm. The concentration of 30 µg/ ml showed the maximum absorbance of 0.903.

10 mg of ascorbic acid was dissolved in 10 mL of methanol. From this stock solution dilutions were made to obtain concentrations of 10, 20, 30, 40

µg/mL. 1 mL from each of these solutions was taken in different volumetric flasks to which 1 mL of DPPH solution (300 µg/ mL concentration) was added and volume was made up to 10 mL. The absorbance was recorded for these dilutions at 516 ηm after duration of 30 min.

The test solutions were prepared in similar manner as that of standard Ascorbic acid and the absorbance was recorded at 516 nm after duration of 30 mins.

Table 5: Antioxidant Activity

Comp Code	% inhibition			
	10µg/ml	20µg/ml	30µg/ml	40µg/ml
1a	7.20	12.30	37.65	39.42
1b	34.77	34.66	37.65	39.42
1c	7.08	15.61	21.04	22.26
1d	17.71	29.34	30.34	40.86
1e	34.77	37.76	47.17	52.16
1f	18.98	24.67	28.90	34.34
Ascorbic acid	56.03	58.80	65.33	68.55

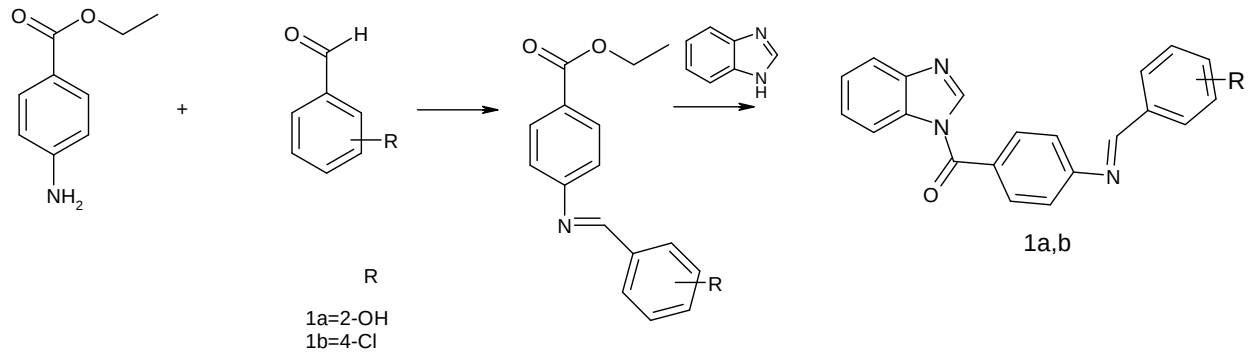
Results & Discussion:

All the compounds synthesized were evaluated for their *invitro* anti-inflammatory activity, it was observed that the compound with phenyl or pyridyl substituted oxadiazole ring fused to benzimidazole moiety through thioacetamide linkage have shown good anti-inflammatory activity, and the compound with phenyl substituted oxadiazole fused to benzimidazole moiety through acetamide linkage has shown least activity, while other derivatives have shown moderate activity.

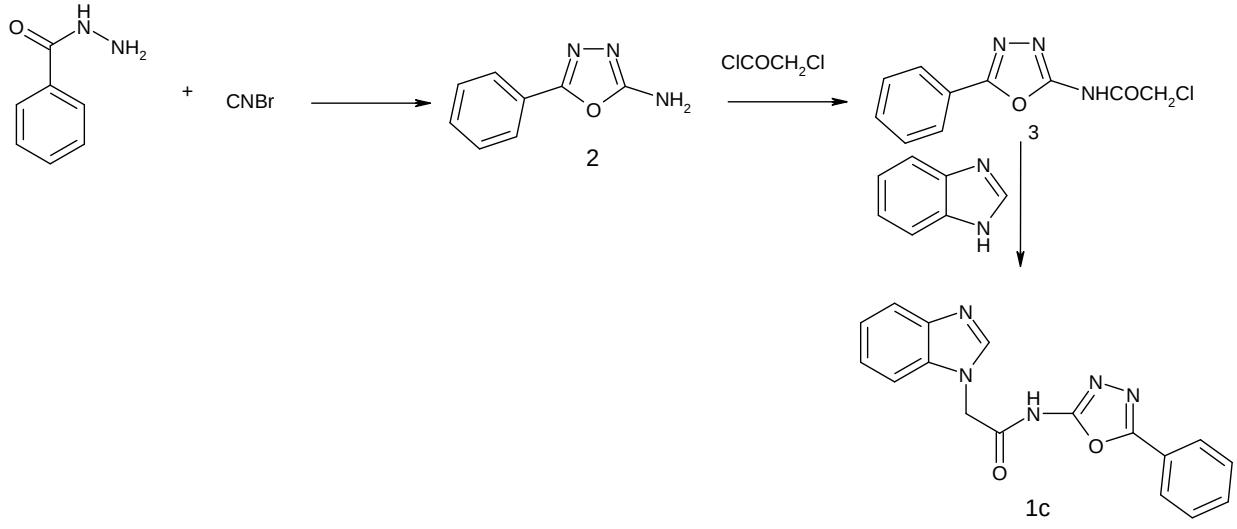
All the compounds were found to show moderate antioxidant activity irrespective of the substitution however the compound with pyridyl substituted oxadiazole has shown good antioxidant activity within the series of compounds synthesized.

Hence the compound with phenyl or pyridyl substituted oxadiazole ring fused to benzimidazole moiety through thioacetamide linkage can be exploited to obtain a pharmacophore as anti-inflammatory agent.

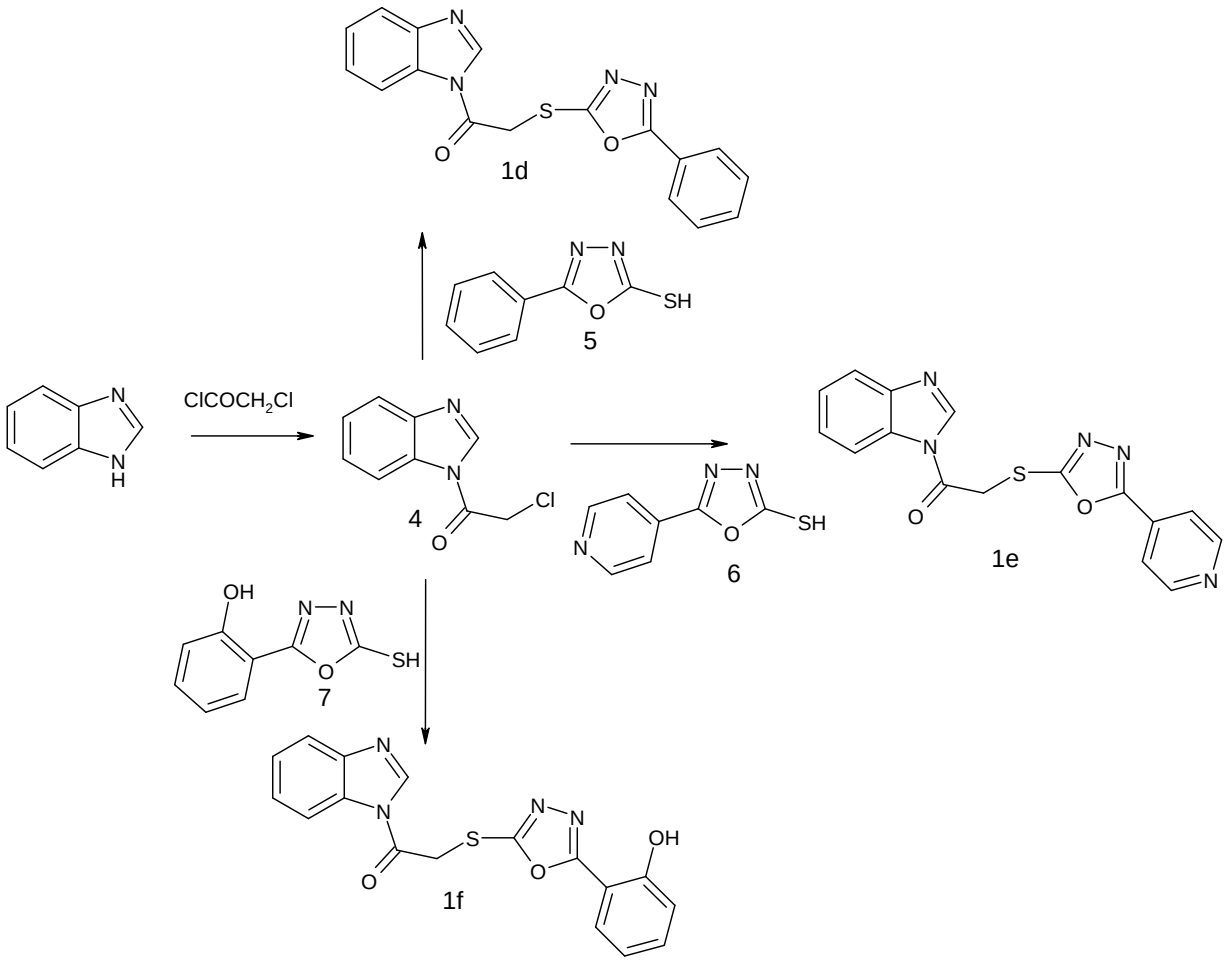
Scheme 1:



Scheme 2:



Scheme 3:



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