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Synthesis, characterization and fungitoxicity of substituted benzimidazoles

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Condensation of *ortho*-phenylenediamine with different substituted carboxylic acids afforded benzimidazoles in the presence of concentrated hydrochloric acid. Synthesized compounds were characterized by their IR, ^{1}H NMR and ^{13}C NMR spectra. The compounds were screened for their fungicidal activity against *Rhizoctonia solani* and *Fusarium moniliforme* by poisoned food technique. All the benzimidazoles exhibited less activity than standard bavistin at all the tested concentrations. Some of the synthesized compounds showed promising to moderate activity.

Keywords: Benzimidazoles, orthophenylenediammine, fungitoxicity, ED_{50}.

Introduction

Heterocyclic compounds are a major class of organic compounds characterized by the fact that atoms in their molecules are joined in a ring containing atleast one atom other than carbon. They exhibit their importance by being an important component of naturally occurring pigments, vitamins and antibiotics. The presence of heteroatoms like nitrogen, oxygen and sulphur has revolutionized the world of synthetic chemistry by exhibiting phenomenal biological activities. The activity is determined by the type and number of heteroatoms, ring size and structure.

One of the primarily important nitrogen containing heterocycle is benzimidazole which is a bicyclic compound having imidazole ring fused to benzene nucleus. The general synthesis of benzimidazoles is by the condensation reaction of 1,2-phenylenediamine with carboxaldehydes[1], carboxylic acids[2] or their derivatives[3,4] such as chlorides, nitriles and orthoesters, under strong acidic conditions and high temperature. The biological significance of benzimidazoles is due to their close relationship with structure of purines. A number of benzimidazole derivatives possessed various biological activities like antimicrobial[5], antiviral[6], antifungal[7], antimalarial[8], antitumour[9], anticancer[10], antihypertensive[11], anti-inflammatory[12] and antioxidant[13] etc.

Keeping in view the biological potential of benzimidazole nucleus, the present investigation was carried out for the synthesis of different 2-substituted benzimidazoles and evaluation of their antifungal activity.

Results and discussion

2-Substituted benzimidazoles were synthesized by reaction of *ortho*-phenylenediamine with different carboxylic acids under strongly acidic conditions to permit cyclisation as depicted in Fig. 1. The products formed were purified by recrystallization, physical data (yield, melting point, R_f, state and colour) was determined and characterized on the basis of elemental analysis and spectral data (IR, ^{1}H NMR and ^{13}C NMR). Compound **1** and **2** took minimum time for reaction i.e. 8 and 5 h respectively.

IR data:

IR spectra of the compounds showed a broad stretching band in the range of 3250–3432 cm^{-1} which was due to N-H of benzimidazole ring. Stretching bands due to C=N and C-N in the range of 1530–1580 cm^{-1} and 1215–1245 cm^{-1} respectively along with C=C (1480–1650 cm^{-1}) and C-H (3050–3180 cm^{-1}) stretch for aromatic ring further confirmed the presence of benzimidazole ring.

^{1}H NMR data:

The formation of benzimidazoles was further confirmed by the ^{1}H NMR spectral data. A broad singlet in the range of δ 9.05–11.04 due to N**H** of benzimidazole ring was observed. Multiplets in the range of δ 6.92 to 7.67 corresponding to

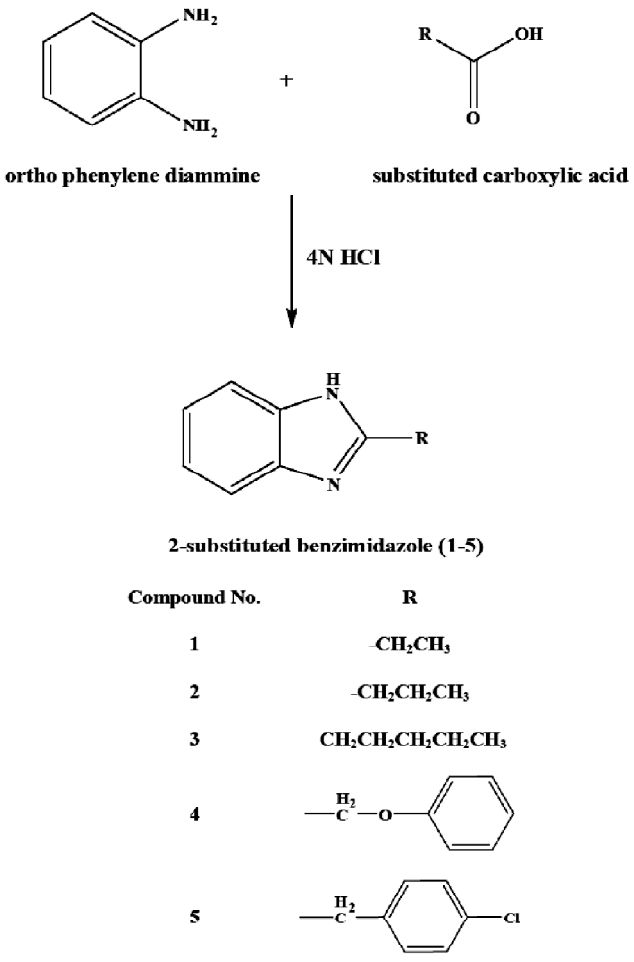

Fig. 1

aromatic protons of the benzimidazole and phenyl rings (**4** and **5**).

^{13}C NMR data:

In ^{13}C NMR, the signal at δ 156.06–159.21 corresponding to N-**C**=N carbon further assured the formation of benzimidazole ring.

Antifungal activity:

Synthesized benzimidazoles were tested for their *in vitro* fungicidal activity against *Fusarium moniliforme* and *Rhizoctonia solani*. The percent inhibition of the compounds against *F. moniliforme* and *R. solani* is presented in Tables 2 and 3 respectively along with ED_{50} values in Tables 4 and 5 respectively. Bavistin was used as standard and DMSO as control against both the test fungi. Compound **4** was found to be most promising against both the pathogenic fungi. Substitution was found to affect the antifungal activity as the compound with the larger hydrophobic aliphatic chain (**3**) was found to be least effective against both the fungi in comparison to those containing aromatic ring (**4** and **5**). The compounds exhibited decrease in antifungal potential with increase in aliphatic chain length against *F. moniliforme*. All the test compounds exhibited antifungal activity at less than 500 ppm against *R. solani*. The ED_{50} values did not show any specific effect of the chain length at 2-substitution against *R. solani*. However none of the compounds showed better control than standard bavistin against both the tested fungi (Fig. 2).

Experimental

General:

Open capillaries methods were used to determine the melting points and are uncorrected. IR, ^{1}H NMR and ^{13}C NMR spectra were got scanned from Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrument Laboratory (CIL), Panjab University, Chandigarh. IR Spectra were recorded using KBr discs on Perkin-Elmer FTIR spectrophotometer with λ_{max} in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrophotometer using TMS as internal reference and $CDCl_3$ as solvent. The chemical shifts were expressed in δ (ppm) values and the

Table 1. Physical parameters of different benzimidazoles

Compd.[a]	Molecular formula	Color	Yield (%)	Melting point (°C)	Time (h)	R_f[b]
1	$C_9H_{10}N_2$	White	78	178–180	8	0.52
2	$C_{10}H_{12}N_2$	Off-white	56	137–139	5	0.45
3	$C_{12}H_{16}N_2$	Off-white	64	130–132	12	0.42
4	$C_{14}H_{12}N_2O$	Light brown	70	158–160	12	0.51
5	$C_{14}H_{11}N_2Cl$	Off-white	42	182–183	15	0.53

[a]All compounds were obtained in solid form.

[b]Mobile phase for thin layer chromatography: 50:50 dichloromethane:ethanol.

Table 2. Percent inhibition (*I*) by benzimidazoles against *Fusarium moniliforme* at different concentrations (μg/mL)

Compd.	Percent inhibition (*I*)				
	50	100	250	500	1000
1	40.01	48.12	61.67	65.35	84.16
2	34.16	35.41	36.25	51.42	65.00
3	17.50	29.37	39.79	48.54	53.12
4	47.5	56.04	67.74	72.32	77.91
5	11.42	31.87	42.08	51.66	55.62
Bavistin	100	100	100	100	100

Table 3. Percent inhibition (*I*) by benzimidazoles against *Rhizoctonia solani* at different concentrations (μg/mL)

Compd.	Percent inhibition (*I*)				
	50	100	250	500	1000
1	19.06	21.37	34.46	75.02	97.52
2	39.58	41.04	49.68	70.83	100
3	28.90	39.53	42.68	52.28	78.12
4	50.8	56.01	65.00	69.32	72.04
5	29.50	40.83	55.20	58.54	71.66
Bavistin	100	100	100	100	100

Table 4. ED_{50} of benzimidazoles against *Fusarium moniliforme*

Compd.	ED_{50} (μg/mL)
1	238
2	598
3	753
4	84
5	445
Bavistin	8

Table 5. ED_{50} of benzimidazoles against *Rhizoctonia solani*

Compd.	ED_{50} (μg/mL)
1	411
2	314
3	482
4	46
5	200
Bavistin	5

abbreviations 's','t', 'q', 'p' and 'm' stand for singlet, triplet, quartet, pentet and multiplet respectively. The purity of the compound was checked on silica gel G coated TLC plates and the visualization was done in iodine chamber. All the compounds gave satisfactory C, H and N analysis that was recorded on Vario EL III Elementor CHNS analyser.

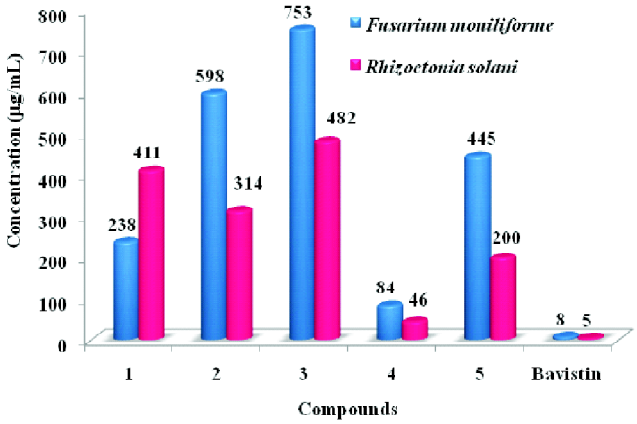

Fig. 2. Antifungal potential (ED_{50}) of benzimidazole derivatives against *Fusarium moniliforme* and *Rhizoctonia solani.*

*General method for synthesis of benzimidazoles (**1-5**):*

In a 250 mL round bottomed flask was taken 30 mL of 4 *N* HCl. To it was added *ortho*-phenylenediamine (0.01 mol) and equimolar amount of substituted carboxylic acid. The reaction mixture was refluxed for required time on heating mantle. The progress of reaction was checked by thin layer chromatography. On completion, the reaction mixture was neutralized with dilute NaOH. Further addition of 1–2 ml of base resulted in the formation of precipitates. The precipitation was completed by stirring the mixture. The solid product formed was filtered and recrystallized from ethanol. The purity of the compound was checked by thin layer chromatography and was indicated by single spot.

Antifungal activity:

Two fungi were selected for bioassay viz. *F. moniliforme* and *R. solani.* A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 7 days) at ambient temperature (25±1°C) for growth. Stock solution (1000 mg/mL) of test compounds was prepared in dimethyl sulphoxide and further dilutions were done (500, 250, 100 and 50 μg/mL) and stored at 4°C for further use. Potato Dextrose Agar media, containing specific concentration of the test compound was poured on to the Petri plates. After solidification, small disc (0.5 cm dia.) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down in the centre of Petri plate. Petri plates were incubated in BOD incubator at 25±1°C. Growth of fungal colony was measured after every 24 h till the fungus in the control plates (containing dimethyl sulfoxide) completely

occupied it. Three replications were maintained for each treatment. The percent growth inhibition over control was calculated as

$$I = \frac{100\ (C - T)}{C}$$

where, I = inhibition percentage, C = growth in control and T = growth in treatment.

*2-Ethyl-1H-benzo[d]imidazole (**1**):* Yield 78%; m.p. 178–180°C; IR (KBr, cm^{-1}): 3427 (N-H str.), 3163 (aromatic C-H str.), 2939 (ν_{as} C-H str.), 2639 (ν_s C-H str.), 1622, 1590, 1541, 1480 (aromatic C=C str.), 1457 (C=N str.), 1242 (C-N str.); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17 (1H, s, N**H**), 7.19–7.54 (4H, m, Ar-**H**), 2.94–3.00 (2H, q, C**H**$_2$CH$_3$, *J* 7.64 Hz) and 1.42–1.46 (3H, t, C**H**$_2$CH$_3$, *J* 7.64 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 156.06 (N=**C**-N), 118.2–138.9 (aromatic carbons), 22.64 (**C**H$_2$CH$_3$), 12.26 (CH$_2$**C**H$_3$).

*2-Propyl-1H-benzo[d]imidazole (**2**):* Yield 56%; m.p. 137–139°C; IR (KBr, cm^{-1}): 3424 (N-H str.), 3095 (aromatic C-H str.), 2975 (ν_{as} C-H str.), 2852 (ν_s C-H str.), 1642, 1601, 1580, 1515 (aromatic C=C str.), 1575 (C=N str.), 1384 (C-H bending, -CH$_3$), 1215 (C-N str.); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.97 (1H, s, N**H**), 7.19–7.65 (4H, m, Ar-**H**), 2.92–2.88 (2H, t, **C**H$_2$CH$_2$CH$_3$, *J* 7.68 Hz), 1.81–1.94 (2H, m, CH$_2$**C**H$_2$CH$_3$) and 0.99–1.03 (3H, t, CH$_2$CH$_2$**C**H$_3$, *J* 7.36 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 151.39 (N=**C**-N), 117.39–139.2 (aromatic carbons), 31.62 (**C**H$_2$CH$_2$CH$_3$), 23.83 (CH$_2$**C**H$_2$CH$_3$), 13.56 (CH$_2$CH$_2$**C**H$_3$).

*2-Pentyl-1H-benzo[d]imidazole (**3**)*: Yield 64%; m.p. 130–132°C; IR (KBr, cm^{-1}): 3432 (N-H str.), 3163 (aromatic C-H str.), 2962 (ν_{as} C-H str.), 2863 (ν_s C-H str.), 1620, 1595, 1542, 1535 (aromatic C=C str.),1545 (C=N str.), 1219 (C-N str.); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 11.04 (1H, s, N**H**), 7.11–7.47 (4H, m, Ar-**H**), 2.84–2.88 (2H, t, **C**H$_2$CH$_2$CH$_2$CH$_2$CH$_3$, *J* 7.68 Hz), 1.74–1.82 (2H, p, CH$_2$**C**H$_2$CH$_2$CH$_2$CH$_3$, *J* 7.68 Hz), 1.18–1.28 (4H, m, CH$_2$CH$_2$**C**H$_2$**C**H$_2$CH$_3$) and 0.73–0.77 (3H, t, CH$_2$CH$_2$CH$_2$CH$_2$**C**H$_3$, *J* 7.08 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 152.61 (N=**C**-N), 115.22–138.91 (aromatic carbons), 30.34 (CH$_2$CH$_2$**C**H$_2$CH$_2$CH$_3$), 29.32 (**C**H$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 23.24 (CH$_2$**C**H$_2$CH$_2$CH$_2$CH$_3$), 22.72 (CH$_2$CH$_2$CH$_2$**C**H$_2$CH$_3$), 13.16 (CH$_2$CH$_2$CH$_2$CH$_2$**C**H$_3$).

*2-(Phenoxymethyl)-1H-benzo[d]imidazole (**4**):* Yield 70%; m.p. 158–160°C; IR (KBr, cm^{-1}): 3372 (N-H str.), 3092 (aromatic C-H str.), 2996 (ν_{as} C-H str.), 2874 (ν_s C-H str.), 1662, 1621, 1590, 1545 (aromatic C=C str.), 1535 (C=N str.), 1227 (C-N str.), 1192 (C-O str.); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.15 (1H, s, N**H**), 6.95–7.60 (9H, m, Ar-**H**), 5.37 (2H, s, **C**H$_2$OC$_6$H$_5$); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 157.68 (N=C-N), 114.65–150.26 (aromatic carbons), 64.27 (**C**H$_2$OC$_6$H$_5$).

*2-(4-Chlorobenzyl)-1H-benzo[d]imidazole (**5**):* Yield 42%; m.p. 182–183°C; IR (KBr, cm^{-1}): 3364 (N-H str.), 3120 (aromatic C-H str.), 2984 (ν_{as} C-H stretch), 2864 (ν_s C-H stretch), 1650, 1611, 1575, 1520 (aromatic C=C str.), 1580 (C=N str.), 1218 (C-N str.), 751 (C-Cl str.); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.06 (1H, s, N**H**), 7.21–7.73 (8H, m, Ar-**H**), 4.25 (2H, s, **C**H$_2$C$_6$H$_4$Cl); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 158.12 (N=**C**-N), 113.6–137.8 (aromatic carbons), 34.71 (**C**H$_2$C$_6$H$_4$Cl).

Conclusions

Different carboxylic acids were treated with *ortho*-phenylenediamine in the presence of hydrochloric acid to yield substituted benzimidazoles (**1-5**). All the benzimidazoles exhibited ED_{50} less than 800 μg/mL and 500 μg/mL against *F. moniliforme* and *R. solani* respectively. Compound **4** was found to be most promising against both the test fungi. The compounds exhibited decrease in antifungal potential with increase in aliphatic chain length against *F. moniliforme*.

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