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Note

Synthesis and *in vitro* study of novel isoxazolyl benzoimidazolyl benzamides, acrylamides and propionamides as antimicrobial agents

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A series of novel 2/3 (1*H*-benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxazolyl)-benzamides, acrylamides and propionamides have been synthesized and the antimicrobial activities are evaluated against two Gram-positive and two Gram-negative bacteria and two plant-pathogenic fungi. Some of the synthesized compounds have showed superior *in vitro* activities as compared to the standard drugs.

Keywords: solid state reaction, isoxazolyl acids, isoxazolyl benzimidazoles, antibacterial activity, anti fungal activity

The benzoimidazole moiety is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity including antiulcer, antitumor, antiviral and antibacterial effects[1-7]. Isoxazoles also constitute an important class of heterocyclic compounds possessing diverse biological activities[8-13]. In addition to being bioactive substances, these compounds find wide applications in synthesis. Due to the continuing increase of antibacterial resistance and the lack of novel antibiotics in the past several decades, it is of great interest to discover novel antibiotics that could combat the antibacterial resistance. This has given an impetus for the synthesis of new isoxazolyl benzoimidazolyl benzamides, acrylamides and propionamides, and evaluation of their biological significance and toxicity.

In continuation of our work on isoxazoles[14-17], the present investigation reports the synthesis and antimicrobial activity of isoxazolyl benzoimidazolyl benzamides, acrylamides and propionamides.

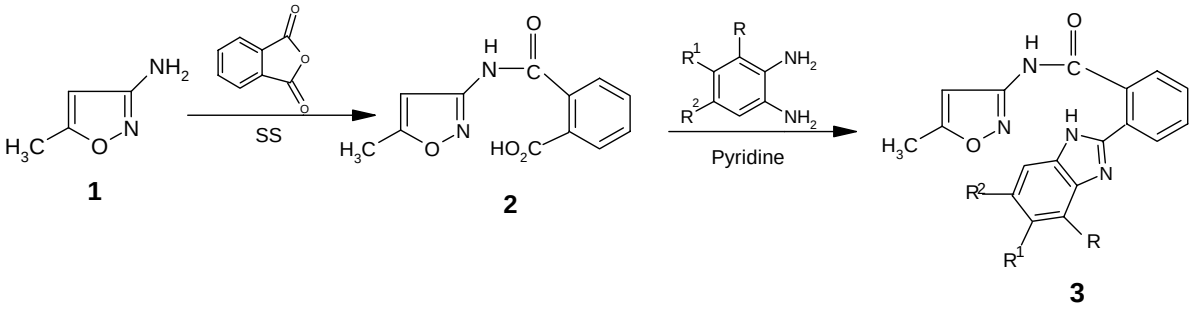
Results and Discussion

3-Amino-5-methylisoxazole **1** was treated at room temperature with phthalic anhydride after grinding both the reactants to a fine powder in a mortar for one hour at ambient temperature (reaction monitored with TLC). It was extracted with aqueous bicarbonate solution. The clear filtrate on neutralization gave a product which was identified as β-(5-methyl-3-isoxazolylamido)-benzoic acid **2** indicating that the anhydride ring had been opened in the solid state **(Scheme Ia).** Similarly, 3-amino-5-methylisoxazole **1** also was treated with maleic anhydride and succinic anhydride under similar conditions, which led to the formation of β-(5-methyl-3-isoxazolylamide)-acrylic acid **4** and propionic acid **6** respectively **(Scheme Ib** and **c)**. The products **2**, **4** and **6** were characterized by elemental analysis **(Table I)** and by IR, ^{1}H NMR and MS spectroscopy (**Table II**).

The foregoing β-(5-methyl-3-isoxazolylamido)-benzoic acid **2** was subjected to cyclocondensation with *o*-phenylenediamine in pyridine to give 2-(1*H*-benzimidazol-2-yl)-*N*-(5-methyl-3-isoxazolyl)benzamides **3 (Scheme Ia)**. Similarly, β-(5-methyl-3-isoxazolylamido)-acrylic acid **4** and propionic acid **6** were cyclized to the corresponding benzimidazoles **5** and **7** respectively by refluxing them in pyridine solvent separately **(Scheme Ib** and **c)**. The structures of **3**, **5** and **7** were identified by analytical **(Table I)** and spectroscopic (IR, ^{1}H NMR and MS) data (**Table II**).

The newly synthesized intermediates, *viz.*, β-(5-methyl-3-isoxazolylamido)-benzoic acid **2**, acrylic acid **4** and propionic acid **6** in their IR spectra exhibited two strong peaks around 3000 and 3200 cm^{-1} due to carboxyl (OH) and amide (NH) groups and two other strong absorptions at 1675 and 1695 cm^{-1} due to amide carbonyl and carboxyl carbonyl groups respectively. ^{1}H NMR spectra of the products **2**,**4** and **6** showed down field signals at δ 9.7 and 11.0 due to amide and carboxyl hydrogens which are D_2O exchangeable. The mass spectra of the products **2**,**4** and **6** agrees very well with the proposed structures.

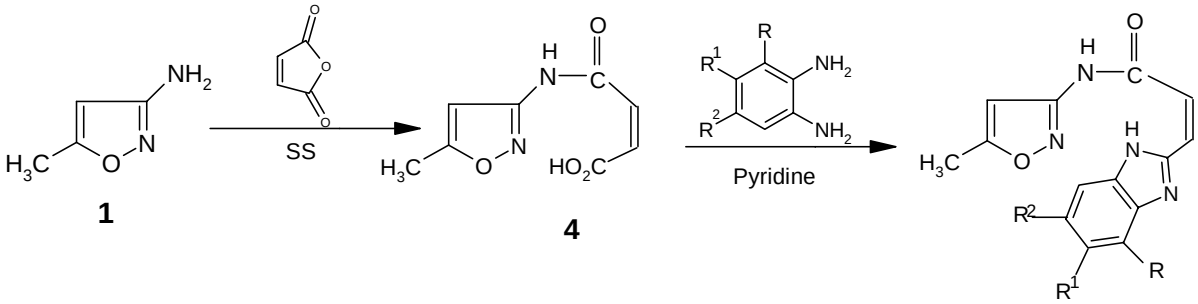
Structures of the title compounds, *viz*., 2/3 (1*H*-benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxazolyl)-benzamides **3**, acrylamides **5** and propionamides **7** were also established by IR, ^{1}H NMR and MS spectra data **(Table II)**. IR spectra of **3**, **5** and **7** showed absorptions around 1685, 3290, 3400 cm^{-1} due to amide carbonyl, amide NH and benzimidazole NH



1 **2** **3**

3a = R, R^1, R^2 = H
3b = R = NO_2, R^1 & R^2 = H
3c = R = H, R^1 & R^2 = Cl

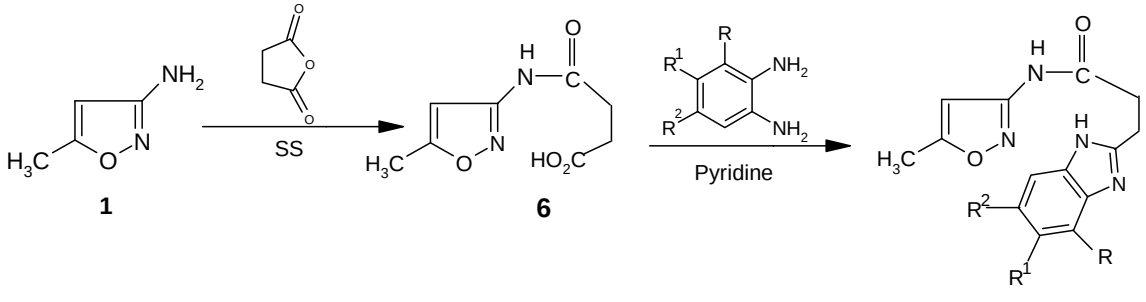
Scheme Ia



1 **4** **5**

5a = R, R^1, R^2 = H
5b = R = NO_2, R^1 & R^2 = H
5c = R = H, R^1 & R^2 = Cl

Scheme Ib



1 **6** **7**

7a = R, R^1, R^2 = H
7b = R = NO_2, R^1 & R^2 = H
7c = R = H, R^1 & R^2 = Cl

Scheme Ic

respectively. ^{1}H NMR spectra of **3**, **5** and **7** conspicuously shown two down field signals at δ 9.7 and 11.0 due to the amide and newly formed benzimidazole ring NH protons respectively. The product structures were further confirmed by their mass spectra.

Antibacterial activity

2/3(1*H*-benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxazolyl)-benzamides **3**, acrylamides **5** and propionamides **7** were screened for their antibacterial activity against two Gram-negative bacteria *Escherichia coli* and *Proteus vulgaris* and two Gram-positive

Table I — Characterization data of compounds **2-7**

No	Compd	R	R^1	R^2	m.p. (^{0}C)	Yield (%)	Mol. Formula	Found (Calcd) (%) C	H	N
1	**2**	--	--	--	165	80	$C_{12}H_{20}N_2O_4$	58.58 (58.53	4.09 4.06	11.39 11.38)
2	**4**	--	--	--	155	80	$C_8H_8N_2O_4$	48.99 (48.97	4.12 4.08	14.29 14.28)
3	**6**	--	--	--	190	85	$C_8H_{10}N_2O_4$	48.49 (48.48	5.09 5.05	14.18 14.14)
4	**3a**	H	H	H	245	85	$C_{18}H_{14}N_4O_2$	67.98 (67.92	4.42 4.40	17.63 17.61)
5	**3b**	NO_2	H	H	220	85	$C_{18}H_{13}N_5O_4$	59.58 (59.50	3.59 3.58	19.29 19.28)
6	**3c**	H	Cl	Cl	238	80	$C_{18}H_{12}N_4O_2Cl_2$	56.02 (55.95	3.15 3.10	14.59 14.50)
7	**5a**	H	H	H	240	85	$C_{14}H_{12}N_4O_2$	62.69 (62.68	4.49 4.47	20.90 20.89)
8	**5b**	NO_2	H	H	205	85	$C_{14}H_{11}N_5O_4$	53.69 (53.67	3.52 3.51	22.38 23.66)
9	**5c**	H	Cl	Cl	218	75	$C_{14}H_{10}N_4O_2Cl_2$	50.09 (50.00	2.99 2.97	16.68 16.66)
10	**7a**	H	H	H	230	80	$C_{14}H_{14}N_4O_2$	62.26 (62.22	5.19 5.18	20.76 20.74)
11	**7b**	NO_2	H	H	200	80	$C_{14}H_{13}N_5O_4$	53.37 (53.33	4.15 4.12	22.26 22.22)
12	**7c**	H	Cl	Cl	215	75	$C_{14}H_{12}N_4O_2Cl$	49.79 (49.70	3.58 3.55	16.58 16.56)

bacteria *Bacillus mycoides* and *Staphylococcus aureus* at 600 and 900 μg/mL concentration using the filter paper disc technique of Vincent and Vincent[18]. The zone of inhibition formed were measured in mm. Streptomycin antibiotic disc (High media SD 181) was used as standard for comparison.

Table III reveals that the compounds of the series **3**, **5** and **7** exhibited appreciable activity against both Gram +ve and –ve bacteria. In this series, the compounds **5a** and **7a** possessing acrylamide and propionamide groups with unsubstituted benzimidazole moieties showed maximum activity by inhibiting growth of all the bacteria to a significant level in comparison with the standard drug streptomycin at the same concentration (900 μg/mL). Hence, they can be exploited for formulation of bacterocides.

Antifungal activity

2/3(1*H*-benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxazolyl)-benzamides **3**, acrylamides **5** and propionamides **7** were also screened for their antifungal activity against two plant pathogens *viz*., *Fusarium oxysporum* and *Drescherla halides* following the glass slide humid chamber technique[19] at 160 μg, 320 μg, 480 μg and 640 μg/mL concentration. Mancozeb (fungicide) was used as the standard for comparison of the activity.

From **Table IV**, it is evident that these compounds exhibit strong fungicidal activity which was lethal even at 480 μg/mL concentration. In this series, the compounds **3a**, **5a**, **7a** and **7b** exhibited remarkable fungitoxicity against both the fungi. This may be due to the presence of the benzimidazole ring-carrying isoxazolyl benzamide, acrylamide and propionamide moieties and also may be due to presence of the nitro group (in compound **7b**). Hence, they can be exploited for formulation of fungicides (**Table IV**).

Experimental Section

All the melting points were determined in open capillary on a cintex melting point apparatus and are

Table II — IR, ^{1}H NMR and MS spectral data of compounds **2-7**

No	Compd	R	R^1	R^2	IR (KBr, cm^{-1})	^{1}H NMR (300 MHz, $CDCl_3$,δ)	MS(M^+)
1	**2**	--	--	--	3285 (NH) 3025 (OH) 1735 (C=O) 1660 (NHCO)	2.4 (s, 3H, CH_3), 6.7 (s, 1H, isoxazole-H), 7.4-7.6 (m, 4H, Ar-H), 7.9 (s, 1H, NH, D_2O exchangeable), 11.0 (s, 1H, OH, D_2O exchangeable)	246
2	**4**	--	--	--	3230 (NH) 3085 (OH) 1710 (C=O) 1630 (NHCO) 975 (C=C)	2.4 (s, 3H, CH_3), 6.2 (d, CH=CH), 6.5 (d, 1H, CH=CH) 6.7 (s, 1H, isoxazole-H), 7.5 (s, 1H, NH, D_2O exchangeable), 11.8 (s, 1H, OH, D_2O exchangeable)	196
3	**6**	--	--	--	3285 (NH) 3025 (OH) 1715 (C=O) 1670 (NHCO)	2.3 (s, 3H, CH_3), 2.5 (m, 4H, CH_2CH_2), 6.8 (s, 1H, isoxazole-H), 7.5 (s, 1H, NH, D_2O exchangeable), 10.6 (s, 1H, OH, D_2O exchangeable)	198
4	**3a**	H	H	H	1708 (NHCO) 3280 (NHCO) 3460 (NH)	2.5 (s, 3H, CH_3), 6.6 (s, 1H, isoxazole-H), 6.8-7.6 (m, 8H, Ar-H), 9.7 (bs, 1H, NHCO, D_2O exchangeable), 11.0 (bs, 1H, benzimidazole-H, D_2O exchangeable)	318
5	**3b**	NO_2	H	H	1698 (NHCO) 3385 (NHCO) 3470 (NH)	2.4 (s,3H,CH_3),6.5 (s,1H, isoxazole-H),6.9-7.2 (m, 7H,Ar-H),9.6 (bs,1H, NHCO, D_2O exchangeable), 11.1 (bs, 1H, benzimidazole-H, D_2O exchangeable)	363
6	**3c**	H	Cl	Cl	1700 (NHCO) 3370 (NHCO) 3465 (NH)	2.3 (s, 3H, CH_3), 6.7 (s, 1H, isoxazole-H), 6.8-7.2 (m, 4H, Ar-H), 7.3 (s, 1H, Ar-H), 7.4 (s, 1H, Ar-H), 9.6 (bs, 1H, NHCO, D_2O exchangeable), 11.2 (bs, 1H, benzimidazole-H, D_2O exchangeable)	386
7	**5a**	H	H	H	1685 (NHCO) 3200 (NHCO) 3350 (NH)	2.3 (s, 3H, CH_3), 6.0 (s, 1H, isoxazole-H), 6.6-6.8 (m, 6H, Ar-H & -CH=CH-),10.3 (bs, 1H, NHCO, D_2O exchangeable), 10.9 (bs, 1H, benzimidazole-H, D_2O exchangeable)	268
8	**5b**	NO_2	H	H	1680 (NHCO) 3225 (NHCO) 3358 (NH)	2.4 (s, 3H, CH_3), 6.1 (s, 1H, isoxazole-H), 6.7-6.9 (m, 5H, Ar-H & -CH=CH-), 10.3 (bs, 1H, NHCO, D_2O exchangeable), 11.0 (bs, 1H, benzimidazole-H, D_2O exchangeable)	313
9	**5c**	H	Cl	Cl	1690 (NHCO) 3290 (NHCO) 3380 (NH)	2.2 (s, 3H, CH_3), 6.1 (s, 1H, isoxazole-H), 6.5-6.9 (m, 4H, Ar-H, -CH=CH-), 10.2 (bs, 1H, NHCO, D_2O exchangeable), 11.2 (bs, 1H, benzimidazole-H, D_2O exchangeable)	336
10	**7a**	H	H	H	1675 (NHCO) 3265 (NHCO) 3400 (NH)	2.3 (s, 3H, CH_3), 2.9 (t, 2H, CH_2-CH_2-), 3.1 (t, 2H, -CH_2-CH_2-), 6.5 (s, 1H, isoxazole-H), 7.0 (m, 2H, Ar-H), 7.3 (m, 2H, Ar-H), 7.8 (bs, 1H, NHCO), 11.0 (bs, 1H, benzimidazole-H)	270
11	**7b**	NO_2	H	H	1660 (NHCO) 3262 (NHCO) 3410 (NH)	2.3 (s, 3H, CH_3), 2.8(t, 2H, CH_2-CH_2-), 3.1 (t, 2H, -CH_2-CH_2-), 6.7 (s, 1H, isoxazole-H), 7.2 (m, 2H, Ar-H), 7.4 (s, 1H, Ar-H), 7.7 (bs, 1H, NHCO, D_2O exchangeable), 11.2 (bs, 1H, benzimidazole-H, D_2O exchangeable)	315
12	**7c**	H	Cl	Cl	1675 (NHCO) 3265 (NHCO) 3450 (NH)	2.4 (s, 3H, CH_3), 2.9(t, 2H, CH_2-CH_2-), 3.3 (t, 2H, -CH_2-CH_2-), 6.7 (s, 1H, isoxazole-H), 7.6 (s, 1H, Ar-H), 7.6 (s, 1H, Ar-H), 7.8 (s, 1H, Ar-H), 7.7 (bs, 1H, NHCO, D_2O exchangeable), 11.1 (bs, 1H, benzimidazole-H, D_2O exchangeable)	338

Table III — Antibacterial screening results of compounds **3**, **5** and **7**

No	Compd	R	R^1	R^2	Conc. (μg/mL)	Zone of inhibition (in mm)			
						E.coli	*P. vulgaris*	*B. mycoides*	*S. aureus*
1	**3a**	H	H	H	600	5.0	6.0	4.0	6.5
					900	9.0	10.0	12.0	13.5
2	**3b**	NO_2	H	H	600	4.5	5.0	3.5	6.0
					900	8.0	10.0	8.5	9.5
3	**3c**	H	Cl	Cl	600	7.5	7.0	8.0	9.0
					900	9.5	11.0	10.0	12.0
4	**5a**	H	H	H	600	6.0	8.0	5.0	6.0
					900	12.8	14.0	11.5	10.0
5	**5b**	NO_2	H	H	600	6.0	8.0	5.8	7.5
					900	10.5	13.5	12.0	11.0
6	**5c**	H	Cl	Cl	600	5.5	6.0	7.5	9.0
					900	8.0	9.0	8.5	10.0
7	**7a**	H	H	H	600	7.0	8.6	5.8	5.6
					900	13.0	15.0	12.5	14.0
8	**7b**	NO_2	H	H	600	5.5	6.0	4.5	6.5
					900	10.5	13.0	9.0	10.0
9	**7c**	H	Cl	Cl	600	7.0	8.0	5.5	7.0
					900	9.5	11.0	11.0	10.5
Streptomycin						9.0	7.5	5.0	3.0
						11.5	10.0	7.0	6.0

uncorrected. Purity of the compounds was checked by TLC. TLC analyses performed on precoated silicagel (E. Merck kieselgel $60F_{254}$) plates and visualization was done by exposing to iodine vapour. IR (KBr) spectra were recorded on a Perkin-Elmer 282 instrument; ^{1}H NMR spectra on a Varian EM-390 spectrometer using TMS as internal reference; and mass spectra on a Varian MAT CH-7 instrument at 70 eV. C, H and N analyses were carried out on Carlo Erba 106 and Perkin – Elmer analysers.

β-(5-methyl-3-isoxazolyl)-benzoic acid 2

3-Amino-5-methyl isoxazole **1** (0.01 mole) and phthalic anhydride (0.01 mole) were mixed well in mortar and pestle. The intimate mixture was ground occasionally for 1hr at room temperature. The reaction-mixture (monitored with TLC) was kept at ambient temperature for another 1hr for completion of the reaction. The resulting solid was extracted with aqueous sodium bicarbonate solution and filtered. The clear filtrate on acidification gave the solid, which was recrystallized from ethyl alcohol to get white crystals.

β-(5-Methyl-3-isoxazolyl)-acrylic acid **4** and propionic acid **6** were prepared similarly following the same reaction conditions as described for **2** (**Table I**).

2-(1*H*-benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxazol-yl) benzamide 3a-c

β-(5-Methyl-3-isoxazolyl)-benzoic acid **2** (0.01 mole) and *o*-phenylenediamine (0.01 mole) were taken in pyridine (30 mL) and the contents were refluxed for 5 hr. The contents after cooling were poured into crushed ice, separated solid was filtered, washed with water. Recrystallization was done with ethyl acetate and benzene.

3-(1*H*-Benzoimidazol-2-yl)-*N*-(5-methyl-3-isoxaz-olyl)-acrylamides **5a-c** and propionamides **7a-c** were prepared similarly following the same reaction conditions as described for **3a-c** (**Table I**).

Table IV — Antifungal screening results of compounds **3, 5** and **7**

No	Compd	R	R^1	R^2	Conc. (μg/mL)	% of spore germination inhibition	
						D. halides	*F. oxysprorum*
1	**3a**	H	H	H	160	270	27.5
					320	59.6	96.0
					480	91.0	100.0
					640	100.0	100.0
2	**3b**	NO_2	H	H	160	17.0	30.0
					320	70.0	97.0
					480	94.5	100.0
					640	100.0	100.0
3	**3c**	H	Cl	Cl	160	15.0	24.0
					320	52.5	46.0
					480	82.5	82.0
					640	100.0	100.0
4	**5a**	H	H	H	160	53.2	62.2
					320	80.3	90.5
					480	94.3	100.0
					640	100.0	100.0
5	**5b**	NO_2	H	H	160	28.6	21.1
					320	54.0	47.6
					480	68.9	72.0
					640	100.0	100.0
6	**5c**	H	Cl	Cl	160	20.0	19.2
					320	39.5	28.0
					480	76.2	66.0
					640	100.0	85.1
7	**7a**	H	H	H	160	27.3	62.0
					320	53.2	95.0
					480	90.3	100.0
					640	100.0	100.0
8	**7b**	NO_2	H	H	160	80.5	55.5
					320	100.0	79.0
					480	100.0	95.8
					640	100.0	100.0
9	**7c**	H	Cl	Cl	160	17.3	30.5
					320	36.6	49.8
					480	58.2	79.0
					640	100.0	86.0

Conclusion

Synthesis of novel isoxazolyl benzoimidazolyl benzamides, acrylamides and propionamides have been achieved in a two-step process, in which the first step is a solid state reaction. The antimicrobial screening results indicated that the compounds **5a** and **7a** are found to be active agents and can be exploited for formulation of bacterocides and fungicides.

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