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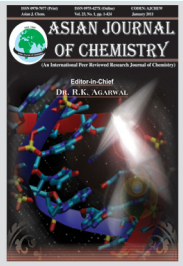

Synthesis and Biological Activity of Pyrazolidine-3,5-Dione Substituted Benzimidazole Derivatives

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Condensation of *o*-phenylene diamine with chloro acetic acid gave 2-chloromethyl benzimdazole, which undergoes halide replacement with phenylhydrazines to give the corresponding N,N'-disubstituted hydrazines. Later these compounds were treated with diethyl malonate in presence of acetic acid to get the pyrazolidine-3,5-dione substituted benzimidazole derivatives. The synthesized compounds were subjected to microbiological screening and *in vitro* antiinflammatory activity.

Key Words: 2-Chloromethyl benzimidazole, Pyrazolidine-3,5-dione, Antimicrobial activity, Antiinflammatory activity.

INTRODUCTION

Benzimidazole derivatives are an important class of nitrogen containing heterocycles and were reported to possess a wide spectrum of biological properties such as antibacterial, analgesic, antiinflammatory, antifungal and antimalarial activities[1,2]. Although a number of drugs are available in the market, but thirst for discovering new antimicrobial drugs with better pharmacokinetic profile and lesser toxicity has become main objectives in the field of medicinal chemistry due to fast development of microbial resistance towards the existing molecules. Despite a number of drugs being in clinical use, search for new NSAIDS is still relevant because the existing molecules suffer from the drawback of adverse effects such as gastric ulceration, inhibition of platelet function, alterations in the renal function, hypersensitivity reactions, *etc*.

Research on benzimidazole and pyrazolidine-3,5-dione and their synthetic analogs have revealed to possess various pharmacological activities along with wide range of antimicrobial activity. It is our interest to synthesize some new 2-chloromethyl benzimidazole derivatives containing pyrazolidine-3,5-dione moiety and evaluate their antimicrobial and *in vitro* antiinflammatory activity. The synthesized compounds have shown satisfactory spectral data which are in conformity of the proposed structures. All the synthesized compounds were screened for antimicrobial activity.

EXPERIMENTAL

Melting points of the synthesized compounds were determined by open capillary method and were uncorrected. IR spectral analysis was carried out using FTIR-8400S, Shimadzu at M.S. Ramaiah College of Pharmacy, Bangalore. ^{1}H NMR spectral data was obtained from Indian Institute of Sciences, Bangalore. The instrument used was amx-400 and the solvent used was deuterated chloroform. The mass spectral data were recorded from LCMS 2010A, Shimadzu provided by UWIN Global Services, Bangalore.

General procedure for preparation of phenyl hydrazines[3]: To a solution of hydrazine hydrate (0.2 mol, 10 mL), hydrochloric acid (10 mL) was added dropwise in such a manner that the temperature of solution was maintained at 5-10 ºC, followed by ethylene glycol (40 mL) and then substituted aniline (0.087 mol) was added. The mixture was refluxed for 2 h, cooled to room temperature. The separated solid was filtered, dried and recrystallized from ethanol. The yields ranged from 50-65 %.

General procedure for synthesis of substituted 2-(chloromethyl)-1*H*-benzimidazole[4-6]: The *o*-phenylene diamine (0.01 mol) was dissolved in 4 N HCl and chloroacetic acid (0.01 mol) was added. The mixture was refluxed for 4 h, cooled and on neutralization with sodium bicarbonate, the product was precipitated. It was filtered, washed with water, dried and recrystallized from ethyl acetate or aqueous ethanol. The yields ranged from 30-60 %. The spectral data is given below:

IR (KBr, ν_{max}, cm^{-1}): 3249, 3213 (N-H *str.*), 1512 (N-H bend), 3056, 3008 (Ar, C-H *str.*), 1469, 1443 (C=C *str.*), 2950 (CH_2 *str.*), 819 (C-Cl); ^{1}H NMR (MeOD): 3.5 (2H, CH_2), 12.0 (1H, NH benzimidazole), 7.3 (2H Ar-benzimidazole), 7.6 (2H, Ar-benzimidazole). Mass: m/e-167 (M^+), 169 (M+2) and other important peaks are 149, 119.

General procedure for synthesis of 2-[(2-phenylhydrazinyl)methyl}-1*H*-benzimidazole[7]: To the ethanolic solution of 2-chloromethylbenzimidazoles (0.02 mol) phenylhydrazine (0.0217 mol) were added and it was refluxed for 5 h. Hot mixture was poured in crushed ice with constant stirring. Separated solid was filtered, dried and recrystallized from ethanol. The yields ranged from 45-65 %. The spectral data is given as: IR (KBr, ν_{max}, cm^{-1}): 3487, 3404 (N-H *str.*), 1510 (N-H bend), 3053 (Ar, C-H *str.*), 2887, 2817 (CH_2 *str.*), 1469, 1436 (C=C *str.*). ^1H NMR ($CDCl_3$): 3.6 (2H, CH_2), 4.2 (1H N-H hydrazinyl), 4.5 (1H N-H hydrazinyl), 12.0 (1H, NH Ar-benzimidazole), 8.3 (4H Ar-benzimidazole), 7.8 (5H, phenyl), Mass: m/e-237 (M^+) and other important peaks are 124, 109.

Synthesis of 1-(1*H*-substituted benzimidazol-2-ylmethyl)-2-substituted phenylpyrazolidine-3,5-dione[8]: A mixture of ethanolic solutions of substituted 2-[(2-phenylhydrazinyl) methyl}-1*H*-benzimidazoles (0.01 mol) and diethyl malonate (1.6 mL, 0.01 mol) to which 2-3 drops of glacial acetic acid were added, was refluxed for 6 h. The resulting liquid was allowed for slow evaporation in a china dish. After few days crystals of the product appeared. The product was recrystallized from ethanol. Their purity was ascertained from TLC [silica gel G plates; mobile phase-benzene:ethyl acetate]. The yields ranged from 45-65 %. The spectral data is given as:

3a: IR (KBr, ν_{max}, cm^{-1}): 3280 (N-H *str.*), 3091, 3062 (Ar, C-H *str.*), 2945, 2840 (CH_2 *str.*), 1685, 1587 (C=O *str.*), 1506 (N-H bend), 1461 (C=C *str.*). ^1H NMR (MeOD): 3.8 (2H, CH_2), 4.3 (2H CH_2 pyrazolidine-3,5-dione), 12.0 (1H, NH benzimidazole), 7.4-8.3 (4H Ar-benzimidazole), 6.9-7.3 (5H, phenyl) Mass: m/e- 306 (M^+).

3b: IR (KBr, ν_{max}, cm^{-1}): 3298, 3263, 3193 (N-H *str.*), 3072, 3053 (Ar, C-H *str.*), 2989, 2904 (CH_2 *str.*), 1704, 1672 (C=O *str.*), 1535 (N-H bend), 1477, 1440 (C=C *str.*), 1404, 1346 (N=O *str.*), 856 (C-N bend). ^1H NMR ($CDCl_3$): 3.8 (2H, CH_2), 4.3 (2H CH_2 pyrazolidine-3,5-dione), 11.9 (1H, NH benzimidazole), 7.5 (4H Ar-benzimidazole), 7.8-8.2 (4H, phenyl), Mass: m/e- 352 (M+1), 353 (M+2).

3c: IR (KBr, ν_{max}, cm^{-1}): 3299, 3263 (N-H *str.*), 3072, 3053 (Ar, C-H *str.*), 2989, 2800 (CH_2 *str.*), 1706, 1672 (C=O *str.*), 1537 (N-H bend), 1515 (C=C *str.*), 1406, 1321 (N=O *str.*), 856 (C-N bend). ^1H NMR ($CDCl_3$): 3.6 (2H, CH_2), 4.4 (2H CH_2 pyrazolidine-3,5-dione), 12.0 (1H, NH benzimidazole), 7.4-8.3 (4H Ar-benzimidazole), 8.3 (3H, phenyl), Mass: m/e- 398 (M+2) and other important peaks are 269, 171, 139, 122.

3d: IR (KBr, ν_{max}, cm^{-1}): 3336 (N-H *str.*), 3043 (Ar, C-H *str.*), 2983, 2908 (CH_2 *str.*), 1704, 1683 (C=O *str.*), 1596 (N-H bend), 1527, 1477 (C=C *str.*), 775 (C-Cl *str.*). ^1H NMR (MeOD): 3.2 (2H, CH_2), 3.7 (2H CH_2 pyrazolidine-3,5-dione) 12.0 (1H, NH benzimidazole), 6.7 (4H Ar-benzimidazole), 8.0 (4H, phenyl), Mass: m/e-341 (M^+), 342 (M+1) and other important peaks are 203.

3e: IR (KBr, ν_{max}, cm^{-1}): 3348 (N-H *str.*), 3060, 3010 (Ar, C-H *str.*), 2970, 2840 (CH_2 *str.*), 1683,1587 (C=O *str.*), 1506 (N-H bend), 1458 (C=C *str.*), 1423, 1330 (N=O *str.*), 846 (C-N bend). ^1H NMR (MeOD): 2.0 (2H, CH_2), 4.2 (2H CH_2 pyrazolidine-3,5-dione), 12.1 (1H, NH benzimidazole), 8.1 (3H Ar-benzimidazole), 6.7 (5H, phenyl), Mass: m/e-352 (M+1), 353 (M+2).

3f: IR (KBr, ν_{max}, cm^{-1}): 3330 (N-H *str.*), 3087, 3078 (Ar, C-H *str.*), 2927, 2840 (CH_2 *str.*), 1666, 1604 (C=O *str.*), 1508 (N-H bend), 1454 (C=C *str.*), 1415, 1336 (N=O *str.*), 845 (C-N bend). ^1H NMR ($CDCl_3$): 2.9 (2H, CH_2), 4.6 (2H CH_2 pyrazolidine-3,5-dione), 12.0 (1H, NH benzimidazole), 7.4 (3H Ar-benzimidazole), 7.5-7.8 (4H, phenyl), Mass: m/e-396 (M^+), 397 (M^+1) and other important peaks are 172, 105, 89.

3g: IR (KBr, ν_{max}, cm^{-1}): 3350, 3230 (N-H *str.*), 3112 (Ar, C-H *str.*), 2975, 2869 (CH_2 *str.*), 1691, 1643 (C=O *str.*), 1589 (N-H bend), 1487, 1452 (C=C *str.*), 1431, 1380 (N=O *str.*, 850 (C-N bend). ^1H NMR (MeOD): 6 (2H, CH_2), 4.4 (2H CH_2 pyrazolidine-3,5-dione), 12.0 (1H, NH benzimidazole), 7.3-8.1 (3H Ar-benzimidazole), 8.4 (3H, phenyl), Mass: m/e-440 (M-1), 441 (M^+).

3h: IR (KBr, ν_{max}, cm^{-1}): 3298, 3263 (N-H *str.*), 3072, 3053 (Ar, C-H *str.*), 2989, 2869 (CH_2 *str.*), 1704, 1672 (C=O *str.*), 1535 (N-H bend), 1477, 1440 (C=C *str.*), 1404, 1346 (N=O *str.*), 856 (C-N bend), 769 (C-Cl *str.*). ^1H NMR ($CDCl_3$): 3.5 (2H, CH_2), 4.0 (2H CH_2 pyrazolidine-3,5-dione), 12.0 (1H, NH benzimidazole), 7.9 (3H Ar-benzimidazole), 8.6 (4H, phenyl), Mass: m/e-386 (M^+), 387 (M+1) and other important peaks are 264.

Physical data of the synthesized compounds are given in the Table-1.

Antimicrobial activity[9]

Antibacterial activity: Antibacterial activity of the synthesized compounds was determined by the cup-plate method against the gram-positive organisms *Staphylococcus aureus*, *Enterococci* and gram-negative organisms *Escherichia coli*, *Shigella* species at 40 µg/mL concentration. The bacteria were subcultured on Nutrient Agar medium. The petri dishes were incubated at 37 ºC for 24 h. Standard antibacterial drugs were also screened under similar conditions for comparison. 0.1 mL of each of norfloxacin (300 µg/mL) and gatifloxacin (300 µg/mL) were used as standards for other microorganisms.

Antifungal activity: The antifungal activity of the synthesized compounds was carried out against the fungi *Aspergillus niger* and *Aspergillus flavus* at 40 µg/mL concentration. The fungi were subcultured in Sabouraud's Dextrose Agar medium. The fungal susceptibility testing was done by cup-plate method using clotrimazole (300 µg/mL) and amphotericin B (1000 units/mL) as standards. The petri dishes were incubated for 48 h at 25 ºC.

Antiinflammatory activity[10]: Denaturation of proteins is one of the causes of inflammation. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. A number of antiinflammatory drugs are known to inhibit the denaturation of proteins. Mizushima and other have employed protein denaturation as *in vitro* screening model for antiinflammatory compounds.

The test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). The final concentration of DMF in all solutions was less than 2.5 %.

Test solution (1 mL) containing different concentration of drug was mixed with 1 mL of 1 mg/mL albumin solution in

TABLE-1
PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS

Compd.	Chemical name	m.f.	m.w. (g)	m.p. (ºC)	Yield (%)
1a	2-(Chloromethyl)-1*H*-benzimidazole	$C_8H_7N_2Cl$	166.61	145-147	31.21
2a	2-[(2-Phenylhydrazinyl)methyl]-1*H*-benzimidazole	$C_{14}H_{14}N_4$	238.29	85-87	54.56
3a	1-(1*H*-Benzimidazol-2-ylmethyl)-2-phenylpyrazolidine-3,5-dione	$C_{17}H_{14}O_2N_4$	306.33	165-167	50.32
3b	1-(1*H*-Benzimidazol-2-ylmethyl)-2-(4-nitrophenyl)pyrazolidine-3,5-dione	$C_{17}H_{13}O_4N_5$	351.32	153-156	60.39
3c	1-(1*H*-Benzimidazol-2-ylmethyl)-2-(2,4-dinitrophenyl)pyrazolidine-3,5-dione	$C_{17}H_{12}O_6N_6$	396.32	180-182	39.89
3d	1-(1*H*-Benzimidazol-2-ylmethyl)-2-(4-chlorophenyl)pyrazolidine-3,5-dione	$C_{17}H_{13}O_2N_4Cl$	340.77	192-196	76.99
3e	1-[(6-Nitro-1*H*-benzimidazol-2-yl)methyl]-2-phenylpyrazolidine-3,5-dione	$C_{17}H_{13}O_4N_5$	351.32	105-107	66.11
3f	1-[(6-Nitro-1*H*-benzimidazol-2-yl)methyl]-2-(4-nitrophenyl)pyrazolidine-3,5-dione	$C_{17}H_{12}O_6N_6$	396.32	130-132	32.32
3g	1-(2,4-Dinitrophenyl)-2-[(6-nitro-1*H*-benzimidazol-2-yl)methyl]pyrazolidine-3,5-dione	$C_{17}H_{11}O_8N_7$	441.32	86-89	38.10
3h	1-(4-Chlorophenyl)-2-[(6-nitro-1*H*-benzimidazol-2-yl)methyl]pyrazolidine-3,5-dione	$C_{17}H_{12}O_4N_5Cl$	385.77	148-150	37.82

phosphate buffer and incubated at 27 ± 1 ºC for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 ºC in water bath for 10 min after cooling, the turbidity was measured at 660 nm in spectrophotometer. The percentage inhibition of denaturation was calculated from control where no drug was added and compared against standard (ibuprofen).

RESULTS AND DISCUSSION

2-Chloromethyl benzimidazoles were prepared by condensation of *o*-phenylene diamines with chloroacetic acid. 2-[(2-Phenyl hydrazinyl)methyl]-1*H*-benzimidazole (**2a-2h**) were prepared by the halide replacement of substituted 2-chloromethyl benzimidazole with phenyl hydrazinyl ring. 1-(1*H*-Benzimidazol-2-ylmethyl)-2-phenylpyrazolidine-3,5-dione derivatives (**3a-3h**) were prepared by condensation reaction of substituted 2-[(2-phenylhydrazinyl) methyl]-1*H*-benzimidazole and diethyl malonate.

Antimicrobial activity

Antibacterial activity: The antibacterial activity of newly synthesized benzimidazole derivatives has been evaluated against Gram positive *Staphylococcus aureus* and *Enterobacter cocci* and Gram negative *Escherichia coli* and *Shigella* species by disc diffusion method. The standards used are norfloxacin and gatifloxacin. The antibacterial data is given in the Table-2.

Antifungal activity

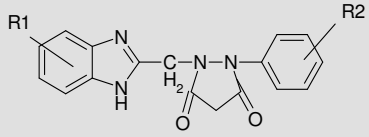
***Aspergillus niger* and *Aspergillus flavus*:** The antifungal activity of newly synthesized benzimidazole derivatives have been evaluated against *Aspergillus niger* and *Aspergillus flavus* the standard used is clotrimazole and amphotericin B. The antifungal data is given in the Table-3.

Antiinflammatory activity: The *in vitro* antiinflammatory activity was performed by adopting the inhibition of bovine serum albumin denaturation method. The standard used is ibuprofen.

The percentage inhibition of bovine serum albumin denaturation of the compound **3a** at the concentration 0.2 mg/mL, 0.4, 0.6, 0.8 and 1.0 mg/mL are 25.22, 33.59, 46.88, 54.58 and 69.17 %, respectively.

All the compounds inhibited the denaturation of serum albumin but **3b**, **3e**, **3c** and **3f** are comparable with standard ibuprofen. The antiinflammatory data is given in the Table-4.

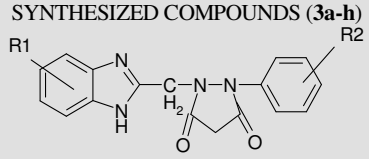
TABLE-2
ANTIBACTERIAL ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS (**3a-h**)



Compd.	R^1	R^2	Antibacterial activity Zone of inhibition (mm) *S. aureus* (Gram + ve)	*Entrococci* (Gram +ve)	*E. coli* (Gram-ve)	*Shigella* (Gram-ve)
3a	H	H	12	08	09	06
3b	H	-4-NO_2	11	10	10	11
3c	H	-2,4-NO_2	13	10	14	13
3d	H	-4Cl	16	10	12	06
3e	6-NO_2	H	18	12	12	08
3f	6-NO_2	-4-NO_2	18	13	14	10
3g	6-NO_2	-2,4-NO_2	17	13	15	06
3h	6-NO_2	-4Cl	14	06	12	05
Norfloxacin	-	-	22	20	25	16
Gatifloxacin	-	-	24	20	24	18
Control (DMF)	-	-	NI	NI	NI	NI

NOTE = Average zone diameter in mm of triplicates

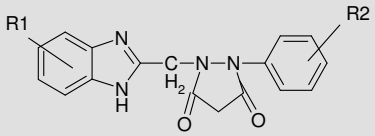
TABLE-3
ANTIFUNGAL ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS (**3a-h**)



Compd.	R^1	R^2	Antifungal activity Zone of inhibition (mm) *Aspergillus niger*	*Aspergillus flavus*
3a	H	H	06	09
3b	H	-4-NO_2	09	10
3c	H	-2,4-NO_2	07	10
3d	H	-4Cl	10	10
3e	6-NO_2	H	12	14
3f	6-NO_2	-4-NO_2	11	13
3g	6-NO_2	-2,4-NO_2	09	11
3h	6-NO_2	-4Cl	09	11
Clotrimoxazol	–	–	13	17
Amphotericin B	–	–	15	18
Control (DMF)	–	–	NI	NI

NOTE = Average zone diameter in mm of triplicates.

TABLE-4
ANTIINFLAMMATORY ACTIVITY DATA OF THE SYNTHESIZED COMPOUNDS



Compd.	R^1	R^2	Inhibition of denaturation of albumin (%)					
			Concentration (mg/mL)					
			Blank	0.2	0.4	0.6	0.8	1.0
3a	H	H	0	25.22	33.59	46.88	54.58	69.17
3b	H	-4-NO_2	0	30.42	40.57	48.07	67.51	64.87
3c	H	-2,4-NO_2	0	20.84	39.64	49.67	57.57	63.17
3d	H	-4Cl	0	11.42	25.81	35.76	48.74	58.17
3e	6-NO_2	H	0	26.02	35.04	44.79	52.96	63.37
3f	6-NO_2	-4-NO_2	0	11.19	23.39	35.88	49.29	60.63
3g	6-NO_2	-2,4-NO_2	0	18.62	34.87	43.82	51.18	55.24
3h	6-NO_2	-4Cl	0	18.26	26.94	33.25	40.43	49.18
Ibuprofen (std.)	–	–	0	37.72	42.21	53.97	60.02	83.27

Conclusion

The observation of other compounds revealed that the substitution of 6-nitro group in benzimidazole ring and replacement of phenyl ring of pyrazolidine-3,5-dione by 4-nitrophenyl, 2,4-dinitrophenyl increases the antibacterial activity. All compounds have shown antibacterial activity against Gram positive bacteria as well as gram negative bacteria namely *Staphylococcus aureus*, *Enterococci* and *Escherichia coli Shigella* (Gram negative).

Here the 6-nitro derivative of benzimidazole shows good activity against *Aspergillus niger* and *Aspergillus flavus*. The observation of other compounds revealed that the substitution of 6-nitro group in benzimidazole ring and replacement of phenyl ring of pyrazolidine-3,5-dione by 4-nitrophenyl, 2,4-dinitrophenyl increases the activity. All compounds have shown antifungal activity against *Aspergillus niger* and *Aspergillus flavus*.

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