

Synthesis and Biological Evaluation of Some 2-Substituted Benzimidazoles

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Some new 2-substituted benzimidazoles were synthesized by the condensation of various clinically used NSAID's and antibacterial agents with different substituted *o*-phenylenediamine dihydrochlorides in presence of ethylene glycol. The structures of the title compounds were derived from elemental analysis, IR, ¹H NMR and mass spectral data. The antiinflammatory, analgesic, anticonvulsant and antimicrobial activity of these compounds are also reported.

Key Words: NSAID's, Benzimidazole, Antiinflammatory, Analgesic, Anticonvulsant, Antimicrobial.

INTRODUCTION

Various 2-substituted benzimidazoles have been reported to possess a wide variety of biological activities like antiinflammatory¹, analgesic², anthelmintic and antibacterial³, antiviral⁴, antifungal⁵ and anticonvulsant⁶. In view of these properties, some new 2-substituted benzimidazoles were synthesized by the condensation of various clinically used NSAID's and antibacterial agents with different substituted *o*-phenylenediamine dihydrochlorides in presence of ethylene glycol. The structures of the title compounds were derived from elemental analysis, IR, ¹H NMR and mass spectral data. The physical data of title compounds is given in Table-1.

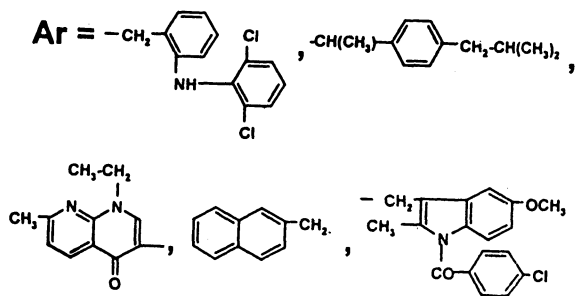
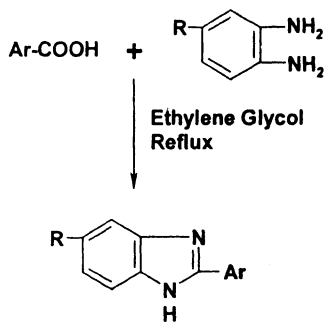
EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. Purity of compounds was checked by TLC on silica gel-G plates and spots were visualized by exposure to the iodine vapours. IR (KBr) spectra were recorded on a Perkin-Elmer 783 spectrophotometer. ¹H NMR (CDCl₃, DMSO-d₆) spectra were recorded on Bruker model DRX-30 NMR spectrophotometer using TMS as internal reference (δ in ppm). The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrophotometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Elemental analysis was performed on Carlo-Erba 1108 analyzer.

TABLE-1
PHYSICAL DATA OF 2-SUBSTITUTED BENZIMIDAZOLES

Compd. No.	R	m.f.	m.w.	m.p. (°C)	Yield (%)	R _f value	Elemental analysis (%), Found (Calcd.)		
							C	H	N
Ia	H	C ₂₀ H ₁₅ N ₃ Cl ₂	368	260	70	0.82	65.21 (65.22)	4.07 (4.10)	11.38 (11.41)
Ib	CH ₃	C ₂₁ H ₁₇ N ₃ Cl ₂	382	270	65	0.80	65.95 (65.97)	4.44 (4.48)	10.96 (10.99)
Ic	NO ₂	C ₂₀ H ₁₄ N ₄ O ₂ Cl ₂	413	250	65	0.75	58.10 (58.12)	3.38 (3.41)	13.51 (13.55)
Id	H	C ₁₉ H ₂₂ N ₂	278	233	70	0.78	81.96 (81.97)	7.93 (8.27)	10.05 (10.06)
Ie	CH ₃	C ₂₀ H ₂₄ N ₂	292	225	60	0.74	82.12 (82.14)	8.22 (8.27)	10.55 (9.58)
If	NO ₂	C ₁₉ H ₂₁ N ₃ O ₂	323	190	75	0.79	70.56 (70.56)	6.51 (6.54)	12.97 (12.99)
Ig	H	C ₁₈ H ₁₆ N ₄ O	304	250	60	0.75	71.01 (71.03)	5.27 (5.29)	18.37 (18.40)
Ih	CH ₃	C ₁₉ H ₁₈ N ₄ O	318	235	70	0.85	71.66 (71.67)	5.67 (5.69)	17.55 (17.59)
Ii	NO ₂	C ₁₈ H ₁₅ N ₅ O ₃	349	215	70	0.83	61.84 (61.85)	4.29 (4.32)	20.04 (20.04)
Ij	H	C ₁₈ H ₁₄ N ₂	258	172	65	0.82	83.66 (83.69)	5.43 (5.46)	10.81 (10.84)
Ik	CH ₃	C ₁₉ H ₁₆ N ₂	272	222	65	0.74	83.77 (83.79)	5.88 (5.92)	10.25 (10.28)
Il	NO ₂	C ₁₈ H ₁₃ N ₃ O ₂	303	150	60	0.78	71.26 (71.27)	4.31 (4.32)	13.83 (13.85)
Im	H	C ₂₃ H ₂₀ N ₃ O ₂ Cl	405	240	65	0.76	68.04 (68.06)	4.93 (4.96)	10.33 (10.35)
In	CH ₃	C ₂₄ H ₂₂ N ₃ O ₂ Cl	419	210	60	0.80	68.62 (68.64)	5.26 (5.28)	9.99 (10.00)
Io	NO ₂	C ₂₃ H ₁₉ N ₃ O ₄ Cl	436	250	60	0.79	63.21 (63.23)	4.35 (4.38)	9.58 (9.61)

*R_f values as determined in methanol : chloroform (95 : 5).



Synthesis of hydrochloride salts of aromatic diamines: 6 g of *o*-phenylenediamine was dissolved in 12 mL of conc. HCl and 8 mL of water containing approximately 0.5 g of tin(II) chloride. The hot solution was treated with 1–2 g of decolourising carbon. The resulting solution was filtered and 10 mL of conc. HCl was added to hot colourless filtrate. It was cooled in a freezing mixture of ice and salt. The colourless crystals of dihydrochloride were collected on a Buchner funnel, washed with small volume of conc. HCl and dried in a vacuum desiccator over sodium hydroxide (yield = 75%).

Synthesis of 2-[(2',6'-dichlorophenyl)amino]benzylbenzimidazole (Ia): Equimolar quantities of *o*-phenylenediamine hydrochloride and diclofenac were refluxed in a round bottom flask containing about 50 mL of ethylene glycol on an oil bath for about 6 h. The reaction mixture was poured on crushed ice and the precipitate so obtained was filtered, dried and recrystallized from ethanol, m.p. 260°C; IR (KBr) cm^{-1} : 3430, 3350, 1595, 1498, 1460, 1365, 1220, 1180, 1105, 1022, 780; $^1\text{H NMR}$ (CDCl_3 , DMSO-d_6) δ ppm: 4.21 (s, 2H, CH_2), 6.88 (m, 4H, Ar—H), 7.20 (m, 4H, Ar—H), 7.58 (m, 3H, Ar—H), 8.20 (s, 1H, NH), 9.12 (s, 1H, NH); MS (m/z): 368 (M^+ , 100%), 369 ($\text{M}^+ + 1$), 370 ($\text{M}^+ + 2$), 331, 307, 279, 214, 206, 167, 154, 136.

2-[(2',6'-Dichlorophenyl)amino]benzyl-5-methylbenzimidazole (Ib): m.p. 270°C; IR (KBr), cm^{-1} : 3415, 3365, 1600, 1550, 1480, 1455, 1365, 1210, 1150, 1035, 850; $^1\text{H NMR}$ (CDCl_3 , DMSO-d_6) δ ppm: 2.50 (s, 3H, CH_3), 4.71 (s, 2H, CH_2), 6.39 (m, 2H, Ar—H), 7.28 (m, 4H, Ar—H), 7.77 (m, 4H, Ar—H), 8.22 (s, 1H, NH), 9.15 (s, 1H, NH).

2-[(2',6'-Dichlorophenyl)amino]benzyl-5-nitro-benzimidazole (Ic): m.p. 250°C; IR (KBr) cm^{-1} : 3420, 3315, 1660, 1610, 1558, 1495, 1460, 1440, 1360, 1240, 1220, 1118, 1105, 880, 795, 750; $^1\text{H NMR}$ (CDCl_3 , DMSO-d_6) δ ppm: 4.55 (s, 2H, CH_2), 6.55 (m, 2H, Ar—H), 7.23 (d, $J = 12$ Hz, 2H, Ar—H), 7.33 (d, $J = 12$ Hz, 2H, Ar—H), 7.75 (m, 4H, Ar—H), 8.15 (s, 1H, NH), 9.25 (s, 1H, NH).

2-[2'-(4'-isobutylphenyl)ethyl]benzimidazole (Id): m.p. 233°C; IR (KBr) cm^{-1} : 3330, 1620, 1580, 1550, 1525, 1450, 1345, 1260, 1210, 1180, 1110, 1045, 984, 913, 850; $^1\text{H NMR}$ (CDCl_3 , DMSO-d_6) δ ppm: 2.50 (bs, 10H, 3XCH_3 , —CH—), 3.22 (bs, 3H, CH_3), 7.75 (m, 4H, Ar—H), 8.18 (m, 4H, Ar—H), 8.20 (s, 1H, NH); MS (m/z): 278 (M^+), 279 ($\text{M}^+ + 1$, 100%), 280 ($\text{M}^+ + 2$), 263, 235, 221, 219, 206, 169, 161, 145, 119, 117.

2-[2'-(4''-Isobutylphenyl)ethyl]-5-methylbenzimidazole (Ie): m.p. 225°C; IR (KBr) cm^{-1} : 3360, 1650, 1540, 1510, 1430, 1380, 1325, 1220, 1185, 1155, 1105, 1055, 975, 913, 845; $^1\text{H NMR}$ (CDCl_3), 7.55 (s, 1H, Ar—H), 7.70 (s, 3H, CH_3), 2.52 (bs, 10H, 3XCH_3 , —CH—), 3.30 (bs, 3H, CH_3), 7.55 (s, 1H, Ar—H), 7.70 (s, 2H, Ar—H), 8.11 (d, $J = 10$ Hz, 1H, Ar—H), 8.19 (d, $J = 10$ Hz, 1H, Ar—H), 8.28 (d, $J = 10$ Hz, 3H, Ar—H, NH);

2-[2'-(4''-Isobutylphenyl)ethyl]-5-nitrobenzimidazole (If): m.p. 190°C; IR (KBr) cm^{-1} : 3450, 1620, 1540, 1480, 1350, 1265, 1180, 1110, 1070, 980, 840; $^1\text{H NMR}$ (CDCl_3 , DMSO-d_6) δ ppm: 2.58 (bs, 10H, 3XCH_3 , DMSO-d_6 δ ppm : 2.25 CH_3), 7.58 (s, 1H, Ar—H), 7.66 (s, 2H, Ar—H), 8.01 (d, $J = 10$ Hz, 1H, Ar—H), 8.10 (d, $J = 10$ Hz, 1H, Ar—H), 8.38 (d, $J = 10$ Hz, 3H, Ar—H, NH);

MS (*m/z*): 323 (M^+), 292, 291, 277, 262, 240, 221, 194, 179 (100%), 178, 177, 161, 148, 119, 104, 90.

2-(1'-Ethyl-1',4'-dihydro-7'-methyl-4'-oxo-naphthyridine-3'-yl)benzimidazole (Ig): m.p. 250°C; IR (KBr) cm^{-1} : 3455, 1710, 1625, 1590, 1555, 1535, 1495, 1260, 1210, 1170, 1125, 1060, 986, 875, 830; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 1.45 (t, $J = 6$ Hz, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.64 (q, 2H, CH_2), 7.16 (t, $J = 8$ Hz, 3H, Ar—H), 7.49 (d, $J = 12$ Hz, 1H, Ar—H), 7.59 (t, $J = 6$ Hz, 1H, Ar—H), 7.68 (t, $J = 8$ Hz, 1H, Ar—H), 8.62 (d, $J = 12$ Hz, 1H, Ar—H), 9.34 (s, 1H, NH); MS (*m/z*): 304 (M^+), 305 ($M^+ + 1$, 100%), 306 ($M^+ + 2$), 276, 277, 219, 154, 136, 119.

2-(1'-Ethyl-1',4'-dihydro-7'-methyl-4'-oxo-naphthyridine-3'-yl)-5-methylbenzimidazole (Ih): m.p. 235°C; IR (KBr) cm^{-1} : 3380, 1715, 1665, 1625, 1580, 1545, 1460, 1366, 1310, 1250, 1180, 1135, 1065, 1010, 975, 910, 855, 790; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 1.42 (t, $J = 8$ Hz, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 4.52 (q, 2H, CH_2), 6.91 (s, 1H, Ar—H), 7.08 (d, $J = 12$ Hz, 2H, Ar—H), 7.21 (d, $J = 12$ Hz, 2H, Ar—H), 7.55 (s, 1H, Ar—H), 8.27 (s, 1H, NH).

2-(1'-Ethyl-1',4'-dihydro-7'-methyl-4'-oxo-naphthyridine-3'-yl)-5-nitrobenzimidazole (Ii): m.p. 215°C; IR (KBr) cm^{-1} : 3410, 1725, 1665, 1615, 1540, 1475, 1420, 1350, 1265, 1220, 1180, 1135, 1090, 1015, 985, 910, 850, 770; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 1.44 (t, $J = 8$ Hz, 3H, CH_3), 2.55 (s, 3H, CH_3), 4.62 (q, 2H, CH_2), 6.98 (s, 1H, Ar—H), 7.15 (d, $J = 12$ Hz, 4H, Ar—H), 7.48 (s, 1H, Ar—H), 8.30 (s, 1H, NH).

2-Naphthylmethylbenzimidazole (Ij): m.p. 172°C; IR (KBr) cm^{-1} : 3410, 1586, 1424, 1318, 1198, 1010, 736; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 4.66 (s, 2H, CH_2), 7.10 (d, $J = 8$ Hz, 1H, Ar—H), 7.12 (d, $J = 8$ Hz, 1H, Ar—H), 7.49 (m, 5H, Ar—H), 7.65 (s, 2H, Ar—H), 7.86 (t, $J = 4$ Hz, 1H, Ar—H), 7.90 (d, $J = 8$ Hz, 1H, Ar—H), 8.21 (s, 1H, NH).

2-Naphthylmethyl-5-methylbenzimidazole (Ik): m.p. 222°C; IR (KBr) cm^{-1} : 3430, 1616, 1430, 1330, 1170, 1040, 986, 840, 736; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 2.25 (s, 3H, CH_3), 2.41 (s, 2H, CH_2), 6.98 (s, 1H, Ar—H), 7.12 (d, $J = 12$ Hz, 4H, Ar—H), 7.35 (s, 1H, Ar—H), 7.78 (m, 4H, Ar—H), 8.29 (s, 1H, NH).

2-Naphthylmethyl-5-nitrobenzimidazole (Il): m.p. 150°C; IR (KBr) cm^{-1} : 3496, 1590, 1488, 1290, 1270, 1230, 1130, 1010, 895, 820; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 2.36 (s, 2H, CH_2), 6.95 (d, $J = 12$ Hz, 1H, Ar—H), 7.26 (d, $J = 8$ Hz, 2H, Ar—H), 7.37 (s, 2H, Ar—H), 7.48 (d, $J = 8$ Hz, 3H, Ar—H), 7.55 (s, 1H, Ar—H), 7.88 (s, 1H, Ar—H), 8.22 (s, 1H, NH).

2-{1'-(4''-Chlorobenzoyl)-2'-methyl-5'-methoxyindole-3'-yl}methylbenzimidazole (Im): m.p. 240°C; IR (KBr) cm^{-1} : 3420, 1640, 1600, 1490, 1460, 1430, 1410, 1360, 1310, 1278, 1225, 1180, 1105, 1040, 1020, 910, 830, 805; 1H NMR ($CDCl_3$, $DMSO-d_6$) δ ppm: 2.37 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 6.55 (d, $J = 12$ Hz, 2H, Ar—H), 6.63 (d, $J = 12$ Hz, 2H, Ar—H), 7.07 (m, 4H, Ar—H), 7.45 (m, 3H, Ar—H), 8.20 (s, 1H, NH).

2-{1'-(4''-Chlorobenzoyl)-2'-methyl-5'-methoxyindole-3'-yl}methyl-5-methylbenzimidazole (In): m.p. 210°C; IR (KBr) cm^{-1} : 3320, 1715, 1665, 1610,

1550, 1525, 1435, 1345, 1270, 1210, 1160, 1095, 1030, 955, 910, 840, 790; ^1H NMR (CDCl_3 , DMSO-d_6) δ ppm: 2.20 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2), 6.66 (s, 1H, Ar—H), 6.77 (s, 1H, Ar—H), 7.10 (d, $J = 12$ Hz, 4H, Ar—H), 7.55 (m, 4H, Ar—H), 8.32 (s, 1H, NH).

2-{1'-(4''Chlorobenzoyl)-2'-methyl-5'-methoxyindole-3'-yl}methyl-5-nitro-benzimidazole (Io): m.p. 250°C ; IR (KBr) cm^{-1} : 3430, 1595, 1498, 1460, 1265, 1220, 1180, 1105, 1022, 780; ^1H NMR (CDCl_3 , DMSO-d_6) δ ppm: 2.38 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.78 (s, 2H, Ar—H), 7.15 (d, $J = 12$ Hz, 4H, Ar—H), 7.66 (m, 4H, Ar—H), 8.27 (s, 1H, NH).

Antimicrobial activity: The *in-vitro* antimicrobial activity was carried out against 24 h old cultures of three bacteria and one fungus. The bacteria used were *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The fungus used was *Candida albicans*. Pure cultures of microorganisms were procured from the cultures maintained at Department of Pathology, Majeedia Hospital, Hamdard Nagar, New Delhi. The antimicrobial activity was performed by cup-plate method⁷. Nutrient agar and Sabouraud agar media were used for antibacterial and antifungal activity respectively. The compounds were tested at a concentration of 50 $\mu\text{g/mL}$ in dimethylformamide using Amikacin and Fluconazole as standard for antibacterial and antifungal activity respectively. Inhibition was recorded by measuring the diameter of the inhibition zone after 24 h for bacteria and 48 h for fungus. Each experiment was repeated thrice and average of the three independent determinations was recorded.

Antiinflammatory activity: Antiinflammatory activity was performed by carrageenan induced paw oedema test in rats⁸. Indomethacin (25 mg/kg) was administered as standard drug for comparison. The test compounds were tested at dose level of 25 mg/kg. The paw volumes were measured using the mercury displacement technique with the help of a plethysmometer immediately before and after 2 h of carrageenan injection.

Analgesic activity: Test for analgesic activity was performed by tail flick technique⁹ using Wistar albino mice (25–35 g) of either sex selected by random sampling technique. Indomethacin (25 mg/kg) was used as standard drug for comparison. The test compounds were administered at a dose of 25 mg/kg. The reaction time was recorded after 1 h of the administration of standard/test compounds.

Anticonvulsant activity: The selected compounds were screened for anti-convulsant activity by maximal electroshock method¹⁰. The compounds were administered at a dose level of 25 mg/kg orally using Phenytoin sodium (25 mg/kg) as standard drug. After 1 h electroshock was applied and the absence of hind limb extensor response after test compound treatment was considered as the protective index. The results were compared with the activity shown by clinically useful anticonvulsant drug, Phenytoin.

The percentage biological activities of title compounds are summarized in Table-2.

TABLE-2
PERCENTAGE BIOLOGICAL ACTIVITIES OF TITLE COMPOUNDS

Compd. No.	% Biological activity						
	<i>S. Aureus</i> *	<i>E. Coli</i> *	<i>P. aeruginosa</i> *	<i>C. Albicans</i> *	Antiinflam- matory activity†	Analgesic activity†	Anticonvul- sant activity†
Ia	55.76	76.55	55.89	77.66	45	58	88
Ib	83.75	69.65	48.88	88.56	56	67	76
Ic	66.66	55.79	55.89	59.65	79	55	35
Id	27.56	48.88	64.43	55.56	83	74	55
Ie	46.34	75.58	68.66	78.56	55	66	84
If	28.66	73.29	47.54	46.78	44	51	67
Ig	67.55	86.66	77.77	76.65	65	48	55
Ih	57.88	44.87	75.98	66.66	39	46	69
Ii	87.77	64.85	63.22	55.55	43	86	47
Ij	58.56	66.66	40.65	67.78	74	85	36
Ik	64.74	80.55	50.56	78.85	54	48	86
Il	39.55	63.99	58.33	75.66	77	65	57
Im	75.55	56.55	56.66	78.65	80	35	76
In	78.56	69.66	68.95	59.78	63	44	55
Io	59.98	67.77	65.55	66.66	54	55	48

No zone of inhibition for dimethylformamide; *average of three independent determinations; †activity w.r.t. control group; Zone of inhibition for amikacin (100%) = 24 mm (*E. coli*), 32 mm (*S. aureus*), 28 mm (*P. aeruginosa*); Zone of inhibition of fluconazole (100%) = 28 mm (*C. albicans*).

RESULTS AND DISCUSSION

The antimicrobial activity of title compounds revealed that **Ii**, **Ig**, **Ig** and **Ib** exhibited highest activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *Candida albicans* respectively. But these activities were less than their respective standards. In case of *S. aureus*, **Ia**, **Ib**, **Ic**, **Ig**, **Ih**, **Ij**, **Ik**, **Im**, **In** and **Io** showed moderate to good activity. Rest of the compounds did not exhibit appreciable activity against this bacterium. For *E. coli*, compounds **Id** and **Ih** showed mild activity while other compounds exhibited moderate to good activity. Similarly, in case of *P. aeruginosa*, **Ib**, **If** and **Ij** exhibited mild activity. All other compounds showed moderate activity except **Ih** that exhibited good activity. Compound **If** exhibited mild antifungal activity against *Candida albicans* and rest of the compounds showed moderate to good activity.

The antiinflammatory activity of the title compounds revealed that **Id** and **Im** exhibited good activity. Mild activity was shown by **Ia**, **If**, **Ih** and **Ii**. Rest compounds showed moderate antiinflammatory activity. Analgesic activity

revealed that **Ii** and **Ij** exhibited highest analgesic activity. **Ig**, **Ih**, **Ik**, **Im** and **In** did not show appreciable activity while rest of the compounds exhibited moderate analgesic activity. The anticonvulsant activity revealed that three compounds **Ia**, **Ie** and **Ik** exhibited very good activity followed by **Ib** and **Im**. All other compounds exhibited mild to moderate anticonvulsant activity. However, these compounds showed lesser antiinflammatory, analgesic and anticonvulsant activity as compared to their respective standards.

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