SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-SUBSTITUTED PHENYL-1-(SUBSTITUTED PIPERAZIN-1-YL) METHYL)-1*H*-BENZO[*D*]IMIDAZOLES

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(Received 24 April 2012) (Accepted 19 December 2012)

ABSTRACT

A series of biologically active benzimidazole derivatives (2a-2n) was synthesized by the reaction of *o*-phenylenediamine with the derivatives of benzoic acid in presence of 4N-HCl followed by the reaction with piperazine and formaldehyde to undergo Mannich reaction. The structures of all the synthesized Mannich bases were characterized by UV, FTIR, ^{1}H NMR, mass spectroscopy and elemental analysis. The compounds were evaluated for their anthelmintic activity by the identification of paralyzing and death time by using mebendazole as standard in the concentration of 2mg/ml. The compounds 2a, 2b, 2e and 2h were found to be most potent for anthelmintic activity. All the compounds were also evaluated for antibacterial activity against gram-positive bacterial strains like *Bacillus subtilis* and *Streptococcus aureus*, and gram-negative bacterial strains like *Escherichia coli* and *Pseudomonas aeruginosa*. The study was performed through disc diffusion method by using Ciprofloxacin as standard in the concentration of 50µg/mL. The compounds 2e, 2h, 2k, 2l and 2m were found to possess significant antibacterial activity.

Keywords: Benzimidazole, Mannich base, Anthelmintic activity, Antibacterial activity

INTRODUCTION

Benzimidazole derivatives are an important class of nitrogen containing heterocycles and which is the most promising heteroaryl moiety has yielded many successful drugs[1-2]. Benzimidazole and its derivatives were found to possess biological activities such as antibacterial[3-9], anticancer[10-11], antidiabetic[12], anthelmintic[13], analgesic, anti-inflammatory[14-15] and antioxidant[16] with substitution at various positions. Benzimidazoles are a group of molecules which have shown potential applications in a variety of pharmacological targets[17]. The synthesis of novel benzimidazole derivatives remains an important focus of medicinal research. Recent observations suggest that substituted benzimidazoles and heterocycles, which are the structural isosters of nucleotides owing fused heterocyclic nuclei in their structures that allow them to interact easily with the biopolymers, possess potential activity in the chemotherapeutic approach in animals. Moreover, these fused heterocycles were distinctively studied for their antitumor, antiviral and antimicrobial activities. An number of heterocyclic compounds of medicinal interest have already been reported from our research laboratory[18-22]. Benzimidazole containing drugs have broadened scope in remedying various dispositions in clinical medicine[23].

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MATERIALS AND METHODS

The chemicals used were procured from Qualigens® Fine Chemicals, Mumbai and Central Drug House (P.) Ltd., New Delhi. All the melting points were determined in open capillary tubes and are uncorrected. Thin layer chromatography using silica gel G (E. Merck) plates was used to access the reactions and purity of synthesized compounds. Satisfactory C, H, N analyses were obtained for all the compounds on a Carlo Erba EA 1108 elemental analyzer. The λ_{max} of the synthesized compounds were scanned on UV-Visible Spectrophotometer

Pharma Spec-1700 (SHIMADZU). IR spectrum of compounds in KBr pellets were recorded on a FTIR-8400 S spectrophotometer (SHIMADZU). ^{1}H NMR spectra of the compounds were recorded on Bruker NMR spectrophotometer at 300 MHz in DMSO-d_6 using TMS as internal standard. Mass spectra were obtained using LC-MS, 2010A (SHIMADZU) using ESI technique.

General Procedure

Synthesis of 2-substituted phenyl-1-(substituted piperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2a-2n)

o-Phenylenediamine (0.02mole) was refluxed with derivatives of benzoic acids (0.02mole) in presence of 4N HCl (20 mL) for 3-5 hrs. The completion of reaction was checked by TLC and then 10% NaOH solution (w/V) was slowly added until the reaction mixture turns alkaline. The reaction mixture was cooled and allowed to stand for 5 minutes to get crude product which was recrystallised to obtain 2-substituted phenyl benzimidazoles (1a-1n). 2-Substituted phenyl benzimidazole (0.01 mole) was dissolved in ethanol (15ml) followed by addition of piperazine derivatives (0.01mole) and formaldehyde solution (40% w/V) (0.015mole) to undergo Mannich reaction. The reactants were refluxed for 6-10 hrs with continuous stirring at 70-75˚C. The completion of reaction was checked by TLC. After completion, the reaction mixture was kept in a refrigerator overnight. The product precipitated out and was filtered, dried and recrystallised from ethanol to give solid compounds (2a-2n).

Analytical and Spectral Data

2-Phenyl-1-((piperazin-1-yl)methyl)-1*H*-benzo[*d*] imidazole (2a)

UV λ_{max} (DMSO)**:** 263.0 nm; IR (KBr)ν_{max}**:** 3328 (N-H stretching, 2°amine), 3054 (aromatic C-H stretching), 2883 (aliphatic C-H stretching), 1657 (aromatic C=N stretching),1593 (aromatic C-C stretching), 1575 (aromatic C=C stretching), 1310 (aromatic C-N stretching), 1153 (aliphatic C-N stretching), 734 cm^{-1} (C-H (monosubstituted benzene) (def)); ^{1}H NMR **(**DMSO-d_6**)** δ (ppm)**:** 1.84 (1H, s, N-H, D_2O exchangeable), 2.22-2.29 (4H, t, N-CH_2), 2.82-2.87 (4H, t, N-CH_2), 4.85 (2H, s, N-CH_2), 6.80-6.82 (2H, t, Ar-H), 6.91-6.95 (1H, t, Ar-H), 7.12-7.16 (2H, t, Ar-H), 7.34-7.38 (2H, d, Ar-H), 7.53-7.55 (2H, d, Ar-H); MS (ESI) m/z [% rel. abundance] : 292 (100) (M^+), 293 (22) $[M+1]^+$, 216 (31), 131(45), 117 (73), 91 (64); elemental analysis calcd for $C_{18}H_{20}N_4$: C, 73.94; H, 6.89; N, 19.16. found: C, 73.91; H, 6.86; N, 19.13.%.

2-(4-Chlorophenyl)-1-((piperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2b)

UV λ_{max} (DMSO)**:** 268.0 nm; IR (KBr)ν_{max}**:** 3326 (N-H stretching, 2°amine), 3062 (aromatic C-H stretching), 2889 (aliphatic C-H stretching), 1665 (aromatic C=N stretching), 1589 (aromatic C-C stretching), 1533 (aromatic C=C stretching), 1285(aromatic C-N stretching), 1174 (aliphatic C-N stretching), 1092 (aromatic C-Cl stretching), 846 cm^{-1} (C-H (*p*-disubstituted benzene)(def)); ^{1}H NMR **(**DMSO-d_6**)** δ(ppm)**:** 1.93 (1H, s, N-H, D_2O

Table I: Physical data of Benzimidazole derivatives 2a-2n

Compd. no.	R_1	R_2	m.p.(°C)	*R_f value	Yield (%)
2a	-H	-H	130-131	0.67	68
2b	4-Cl	-H	185-186	0.61	64
2c	4-NH_2	-H	237-238	0.57	65
2d	3-NH_2	-H	227-228	0.70	66
2e	2-NO_2	-H	230-232	0.77	70
2f	3-OCH_3	-H	230-231	0.56	69
2g	4-CH_3	-CH_3	221-222	0.66	67
2h	2-NO_2	-CH_3	223-224	0.63	72
2i	3-NH_2	-CH_3	181-182	0.58	62
2j	4-NH_2	-CH_3	148-149	0.45	64
2k	4-Cl	-C_2H_5	241-242	0.59	69
2l	2-NO_2	-C_2H_5	142-143	0.67	71
2m	-H	-C_2H_5	123-124	0.70	69
2n	4-CH_3	-C_2H_5	147-148	0.63	67

**Mobile phase : Chloroform : Methanol (9:1)*

Table II: Biological Activity Data of Synthesized Compound 2a-2n

Compound code	Anthelmintic activity *(Phaeritima posthuma)*		Antibacterial activity at 50 µg/ml			
	Mean paralyzing time (min)	Mean death time (min)	*B. subtilis* (NCIM2063)	*S. aureus* (NCIM2079)	*E. coli* (ATCC11775)	*P. aeureginosa* (ATCC10145)
2a	28.39±0.28	33.49±.015	11.83±0.88	10.47±0.25	14.02±0.36	16.43±0.14
2b	23.95±0.14	28.87±0.58	14.80±0.58	15.03±0.45	15.33±0.36	17.63±0.18
2c	32.63±0.15	37.85±0.85	9.73±0.56	10.08±0.47	10.22±0.54	14.23±0.96
2d	33.62±0.25	37.19±0.58	11.73±0.56	11.20±0.85	11.86±0.26	14.56±0.58
2e	21.58±0.65	27.58±0.47	15.06±0.96	15.53±0.88	18.45±0.05	18.23±0.06
2f	32.94±0.35	37.59±0.36	10.07±0.08	9.33±0.25	9.64±0.36	15.08±0.35
2g	36.63±0.46	42.52±0.96	8.53±0.04	8.27±0.08	8.10±0.36	12.43±0.96
2h	20.34±0.25	23.95±0.41	15.53±0.89	13.47±0.85	17.43±0.09	19.66±0.14
2i	27.38±0.58	31.33±0.85	10.47±0.05	11.73±0.25	14.40±0.45	12.23±0.56
2j	28.70±0.63	35.32±0.89	11.56±0.63	12.4±0.25	15.28±0.04	15.29±0.85
2k	25.38±0.69	30.68±0.15	12.36±0.96	15.26±0.08	14.38±0.85	17.16±0.36
2l	26.58±0.36	30.00±0.52	13.16±0.85	15.44±0.14	16.23±0.45	19.43±0.66
2m	21.41±0.25	28.63±0.58	10.53±0.33	14.33±0.77	11.93±0.25	15.43±0.85
2n	40.62±0.85	45.52±0.47	8.47±0.25	12.36±0.41	11.56±0.71	11.26±0.36
*Std.	30.48±0.84	38.28±0.69	14.80±0.96	16.42±0.85	16.25±0.41	18.86±0.09
Blank	-	-	-	-	-	-

**Standard=Mebendazole for anthelmintic activity, Ciprofloxacin for antibacterial activity*

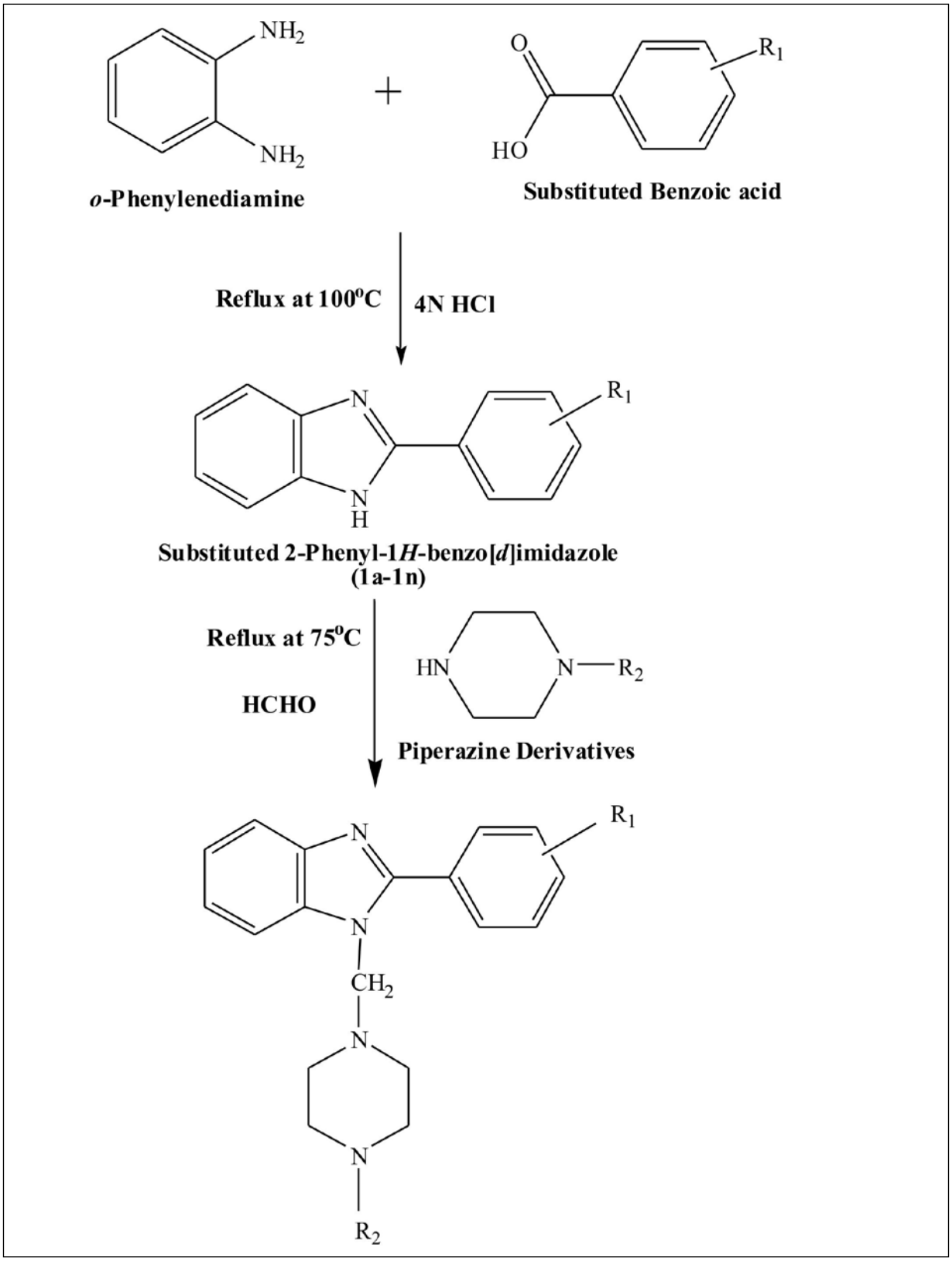
The results of anthelmintic activity were reported as mean paralyzing time and mean death time in min, the results of antibacterial activity were reported as zone of inhibition in mm.. (-: indicates no activity).

Data are given as mean ± S.D (n=3); S.D = Standard deviation

exchangeable), 2.21-2.23 (4H, t, N-CH_2), 2.82-2.89 (4H, t, N-CH_2), 4.83 (2H, s, N-CH_2), 6.81-6.82 (2H, d, Ar-H), 7.13-7.16 (2H, t, Ar-H), 7.35-7.36 (2H, d, Ar-H), 7.53-7.55 (2H, t, Ar-H); MS (ESI) m/z [% rel. abundance] : 326 (100) (M^+), 327 (22) $[M+1]^+$, 328 (34)$[M+2]^+$, 292 (31), 216 (18), 131 (61), 117 (22), 91 (82); elemental analysis calcd for $C_{18}H_{19}N_4Cl$: C, 66.15; H, 5.86; N, 17.14. found: C, 66.13; H, 5.83; N, 17.10.%.

2-(4-Aminophenyl)-1-((piperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole (2c)

UV λ_{max} (DMSO)**:** 285.0 nm; IR (KBr)ν_{max}**:** 3392 (aromatic N-H stretching), 3347 (aliphatic N-H stretching, 2°amine), 3042 (aromatic C-H stretching),2800 (aliphatic C-H stretching), 1665 (aromatic C=N stretching), 1587 (aromatic C-C stretching), 1545 (aromatic C=C stretching), 1282(aromatic C-N stretching), 1172 (aliphatic C-N stretching), 812 cm^{-1} (C-H (*p*-disubstituted benzene) (def)); ^{1}H NMR **(**DMSO-d_6**)** δ (ppm)**:** 1.87 (1H, s, N-H, D_2O exchangeable), 2.41-2.45 (4H, t, N-CH_2), 2.62-2.65 (4H, t, N-CH_2), 4.75 (2H, s, N-CH_2), 6.37 (2H, s, Ar. N-H, D_2O exchangeable),6.53-6.54 (2H, d, Ar-H), 7.21-7.26 (2H, t, Ar-H), 7.32-7.39 (2H, d, Ar-H), 7.71-7.73 (2H, d, Ar-H); MS (ESI) m/z [% rel. abundance] : 307 (100) (M^+), 308 (23) $[M+1]^+$,292 (46), 216 (28), 131 (57), 117 (36), 91 (82); elemental analysis calcd for $C_{18}H_{21}N_5$: C, 70.33;H, 6.89; N, 22.78. Found: C, 70.30; H, 6.86; N, 22.76.%.



Scheme 1 : Substituted 2-phenyl-1-((piperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole (2a-2n)

2-(3-Aminophenyl)-1-((piperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2d)

UV λ_{max} (DMSO)**:** 283.0 nm; IR (KBr)ν_{max}**:** 3347(aromatic N-H stretching), 3228 (aliphatic N-H stretching, 2°amine), 3048 (aromatic C-H stretching), 2933 (aliphatic C-H stretching), 1667 (aromatic C=N stretching), 1592 (aromatic C=C stretching), 1587 (aromatic C-C stretching), 1310(aromatic C-N stretching), 1178 (aliphatic C-N stretching),827 cm^{-1} (C-H (*m*-disubstituted benzene)(def)); ^1H NMR **(**DMSO-d_6**)** δ (ppm)**:** 1.92 (1H, s, N-H, D_2O exchangeable), 2.35-2.38 (4H, t, N-CH_2), 2.71-2.73 (4H, t, N-CH_2), 4.84 (2H, s, N-CH_2), 6.32 (2H, s, Ar. N-H, D_2O exchangeable),6.82-6.84 (2H, d, Ar-H), 6.84-6.89 (1H, t, Ar-H), 7.02 (1H, s, Ar-H), 7.32-7.35 (2H, t, Ar-H), 7.67-7.68 (2H, d, Ar-H); MS (ESI) m/z [% rel. abundance] : 307 (100) (M^+), 308 (23) $[M+1]^+$,292 (44), 216 (30), 131 (74), 117 (52), 91 (88); elemental analysis calcd for $C_{18}H_{21}N_5$: C, 70.33; H, 6.89; N, 22.78. Found: C, 70.30; H, 6.85; N, 22.76.%.

2-(2-Nitrophenyl)-1-((piperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2e)

UV λ_{max} (DMSO)**:** 253.0 nm; IR (KBr)ν_{max}**:**3225 (aliphatic N-H stretching, 2°amine), 3089 (aromatic C-H stretching),2896 (aliphatic C-H stretching), 1650 (aromatic C=N stretching), 1610 (aromatic C=C stretching), 1576 (aromatic C-C stretching), 1282(aromatic C-N stretching), 1166 (aliphatic C-N stretching),756 cm^{-1} (C-H (*o*-disubstituted benzene)(def)); ^1H NMR **(**DMSO-d_6**)** δ (ppm)**:** 1.84 (1H, s, N-H, D_2O exchangeable), 2.41-2.42 (4H, t, N-CH_2), 2.61-2.66 (4H, t, N-CH_2), 4.82 (2H, s, N-CH_2), 7.21-7.22 (2H, t, Ar-H), 7.42-7.48 (1H, t, Ar-H), 7.66-7.68 (2H, d, Ar-H), 7.70-7.72 (1H, d, Ar-H), 7.74-7.78 (1H, t, Ar-H), 8.40-8.42 (1H, d, Ar-H); MS (ESI) m/z [% rel. abundance] : 337 (100) (M^+), 338 (23) $[M+1]^+$,292 (33), 216 (59), 131 (83), 117 (22), 91 (35) elemental analysis calcd for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76. found: C, 64.05; H, 5.65; N, 20.72.%.

2-(3-Methoxyphenyl)-1-((piperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2f)

UV λ_{max} (DMSO)**:** 287.0 nm; IR (KBr)ν_{max}**:**3316 (aliphatic N-H stretching, 2°amine), 3092 (aromatic C-H stretching), 2881(aliphatic C-H stretching), 1638 (aromatic C=N stretching), 1610 (aromatic C=C stretching), 1582 (aromatic C-C stretching), 1282 (aromatic C-N atretching), 1164 (aliphatic C-N stretching), 1078 (aliphatic C-O-C stretching), 789 cm^{-1} (C-H (*m*-disubstituted benzene)(def)); ^1H NMR **(**DMSO-d_6**)** δ(ppm)**:** 1.92 (1H, s, N-H, D_2O exchangeable), 2.45-2.48 (4H, t, N-CH_2), 2.62-2.65 (4H, t, N-CH_2), 3.78 (3H, s, CH_3), 4.86 (2H, s, N-CH_2), 6.72-7.73 (1H, d, Ar-H), 6.87 (1H, s, Ar-H), 7.03-7.07 (1H, t, Ar-H), 7.20-7.22 (1H, d, Ar-H), 7.31-7.33 (2H, t, Ar-H), 7.73-7.75 (2H, d, Ar-H);MS (ESI) m/z [% rel. abundance] : 322 (100) (M^+), 323 (23) $[M+1]^+$,292 (41), 216 (27), 131 (61), 117 (22), 91 (87); elemental analysis calcd for $C_{19}H_{22}N_4O$: C, 70.78; H, 6.88; N, 17.38. Found: C, 70.74; H, 6.86; N, 17.35.%.

2-(4-Methylphenyl)-1-(methylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole (2g)

UV λ_{max} (DMSO)**:** 257.0 nm; IR (KBr)ν_{max}**:**3082 (aromatic C-H stretching), 2977(aliphatic C-H stretching), 1654 (aromatic C=N stretching), 1604 (aromatic C=C stretching), 1571 (aromatic C-C stretching), 1284(aromatic C-N stretching), 1174 (aliphatic C-N stretching), 847 cm^{-1} (C-H (*p*-disubstituted benzene)(def)); ^1H NMR **(**DMSO-d_6**)** δ (ppm)**:** 2.27 (3H, s, CH_3),2.35 (3H, s, CH_3), 2.41-2.46 (4H, t, N-CH_2), 2.55-2.57 (4H, t, N-CH_2), 4.75 (2H, s, CH_2), 7.11-7.12 (2H, d, Ar-H), 7.25-7.27 (2H, t, Ar-H), 7.35-7.38 (2H, d, Ar-H), 7.71-7.73 (2H, d, Ar-H);MS (ESI) m/z [% rel. abundance] : 320 (100) (M^+), 321 (24) $[M+1]^+$,306 (27), 230 (38), 131 (52), 117 (42), 91 (75); elemental analysis calcd for $C_{20}H_{24}N_4$: C, 74.97; H, 7.55; N, 17.48. found: C, 74.94; H, 7.53; N, 17.44.%.

2-(2-Nitrophenyl)-1-(methylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole(2h)

UV λ_{max} (DMSO)**:** 284.0 nm; IR (KBr)ν: 3078 (aromatic C-H stretching),2939 (aliphatic C-H

stretching), 1674 (aromatic C=N stretching), 1592 (aromatic C-C stretching), 1494 (aromatic C=C stretching), 1313(aromatic C-N stretching), 1174 (aliphatic C-N stretching), 769 cm^{-1} (C-H (*o*-disubstituted benzene) (def.));^{1}H NMR **(**DMSO-d_6**)** δ (ppm)**:** 2.48 (3H, s, N-CH_3), 2.61-2.63 (4H, t, N-CH_2), 2.71-2.72 (4H, t, N-CH_2), 4.82 (2H, s, N-CH_2), 7.21-7.22 (2H, t, Ar. CH), 7.42-7.44 (1H, t, Ar. CH), 7.70-7.71 (2H, d, Ar. CH), 7.71-7.72 (1H, t, Ar. CH), 7.78-7.79 (1H, d, Ar.CH), 8.22-8.24 ppm (1H, d, Ar. CH); MS (ESI) m/z [% rel. abundance] : 351 (100) (M^+), 352 (23) $[M+1]^+$, 306(70), 230(38), 131(84), 117(62), 91(48) elemental analysis: calcd for $C_{19}H_{21}N_5O_2$: C, 64.94; H, 6.02; N, 19.93; found: C, 64.91; H, 6.00; N, 19.91%.

2-(3-Aminophenyl)-1-(methylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole(2i)

UV λ_{max} (DMSO): 275.0 nm; IR (KBr) ν:3386 (aromatic N-H stretching), 3068 (aromatic C-H stretching),2975 (aliphatic C-H stretching), 1679 (aromatic C=N Stretching), 1608 (aromatic C=C stretching), 1578 (aromatic C-C stretching), 1286(aromatic C-N stretching), 1176 (aliphatic C-N stretching) 778 cm^{-1} (C-H (*m*-disubstituted benzene) (def.)); ^{1}H NMR **(**DMSO-d_6**)** δ (ppm)**:** 2.28 (3H, s, CH_3), 2.41-2.43 (4H, t, N-CH_2), 2.55-2.59 (4H, t, N-CH_2), 4.38 (2H, s, N-CH_2), 6.32 (2H, s, NH_2), 6.41-6.42 (1H, d, Ar. CH), 6.58 (1H, s, Ar. CH), 6.84-6.85 (1H, d, Ar. CH), 7.02-7.05 (1H, t, Ar. CH), 7.33-7.36 (2H, t, Ar. CH), 7.70-7.71 ppm (2H, d, Ar. CH); MS (ESI) m/z [% rel. abundance]: 321 (100) (M^+), 322 (22) $[M+1]^+$, 306(25), 230(31), 131(52), 117(85), 91(66); elemental analysis: calcd for $C_{19}H_{23}N_5$: C, 71.00; H, 7.21; N, 21.79. found: C, 70.97; H, 7.18; N, 21.76 %.

2-(4-Aminophenyl)-1-(methylpiperazin-1-yl) methyl)-1*H*-benzo[*d*]imidazole (2j)

UV λ_{max} (DMSO): 248.0 nm; IR (KBr) ν:3362 (aromatic N-H stretching), 3071 (aromatic C-H stretching),2952 (aliphatic C-H stretching), 1683 (aromatic C=N Stretching), 1602 (aromatic C-C stretching), 1548 (aromatic C=C stretching), 1317(aromatic C-N stretching), 1182 (aliphatic C-N stretching) 853 cm^{-1} (C-H (*p*-disubstituted benzene) (def.)); ^{1}H NMR **(**DMSO-d_6**):** δ2.31 (3H, s, CH_3), 2.39-2.41 (4H, t, N-CH_2), 2.46-2.48 (4H, t, N-CH_2), 4.72 (2H, s, N-CH_2), 5.12 (2H, s, NH), 6.55-6.56 (2H, d, Ar. CH), 7.23-7.24 (2H, d, Ar. CH),7.31-7.32 (2H, t, Ar. CH), 7.71-7.72 ppm (2H, d, Ar. CH); MS (ESI) m/z [% rel. abundance] : 321 (100) (M^+), 322 (22) $[M+1]^+$, 306(43), 230(28), 131(62), 117(35), 91(87); elemental analysis: calcd for $C_{19}H_{23}N_5$: C, 71.00; H, 7.21; N, 21.79. found: C, 70.97; H, 7.19; N, 21.76 %.

2-(4-Chlorophenyl)-1-(ethylpiperazin-1-yl) methyl)-*1H*-benzo*[d]*imidazole (2k)

UV λ_{max} (DMSO): 256.0 nm; IR (KBr) ν: 3051 (aromatic C-H stretching),2935 (aliphatic C-H stretching), 1677 (aromatic C=N Stretching), 1585 (aromatic C-C stretching), 1508 (aromatic C=C stretching), 1315(aromatic C-N stretching), 1174 (aliphatic C-N stretching), 1091 (aromatic C-Cl stretching), 839 cm^{-1} (C-H (*p*-disubstituted benzene) (def.)); ^{1}H NMR **(**DMSO-d_6**)** δ (ppm)**:** 1.04-1.06 (3H, t, CH_3), 2.40-2.43 (2H, m, N-CH_2), 2.54-2.56 (4H, t, N-CH_2), 2.76-2.78 (4H, t, CH_2), 4.83 (2H, s, N-CH_2), 7.23-7.25 (2H, t, Ar. CH), 7.31-7.32 (2H, d, Ar. CH), 7.47-7.48 (2H, d, Ar. CH),7.70-7.71 ppm (2H, d, Ar. CH); MS (ESI) m/z [% rel. abundance] :354 (100) (M^+), 355 (23) $[M+1]^+$, 356 (35) $[M+2]^+$, 320 (53), 244(78), 131(32), 117(61), 91(44); elemental analysis: calcd for $C_{20}H_{23}N_4Cl$: C, 67.69; H, 6.53; N, 15.79; found: C, 67.66; H, 6.50; N, 15.75 %.

2-(2-Nitrophenyl)-1-(ethylpiperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2l):

UV λ_{max} (DMSO): 264.0 nm; IR (KBr) ν:3089 (aromatic C-H stretching.),2970 (aliphatic C-H stretching.), 1685 (aromatic C=N Stretching), 1598 (aromatic C-C stretching), 1531 (aromatic C=C stretching), 1299(aromatic C-N stretching), 1145 (aliphatic C-N stretching), 741 cm^{-1} (C-H (*o*-disubstituted benzene) (def.)); ^{1}H NMR (DMSO-d_6):δ1.01-1.02 (3H, t, CH_3), 2.25-2.28 (2H, m, N-CH_2), 2.36-2.37 (4H, t, N-CH_2), 2.46-2.48 (4H, t, CH_2), 4.80 (2H, s, N-CH_2), 6.75-6.78 (1H, t, Ar. CH), 7.25-7.28 (2H, t, Ar. CH), 7.44-7.46 (1H, t, Ar. CH),7.70-7.71

(1H, d, Ar. CH), 7.75-7.76 (2H, d, Ar. CH), 8.23-8.25 ppm (1H, d, Ar. CH); MS (ESI) m/z [% rel. abundance]: 365 (100) (M^+), 366 (24) $[M+1]^+$, 320(36), 244(88), 131(22), 117(57), 91(48); elemental analysis: calcd for $C_{20}H_{23}N_5O_2$: C, 65.73; H, 6.34; N, 19.16; found: C, 65.70; H, 6.30; N, 19.13%.

2-Phenyl-1-(ethylpiperazin-1-yl)methyl)-1*H*-benzo[*d*]imidazole (2m):

UV λ_{max} (DMSO): 271.0 nm; IR (KBr)ν:3099 (aromatic C-H stretching.),2925 (aliphatic C-H stretching), 1687 (aromatic C=N stretching), 1593 (aromatic C=C stretching), 1574 (aromatic C-C stretching), 1325(aromatic C-N stretching), 1180 (aliphatic C-N stretching), 729 cm^{-1} (C-H (monosubstituted benzene) (def.)); ^{1}H NMR (DMSO-d_6): δ1.11-1.12 (3H, t, CH_3), 2.30-2.32 (2H, m, N-CH_2), 2.42-2.43 (4H, t, N-CH_2), 2.46-2.48 (4H, t, CH_2), 4.82 (2H, s, N-CH_2), 7.21-7.22 (1H, t, Ar. CH), 7.25-7.27 (2H, t, Ar. CH), 7.31-7.32 (2H, t, Ar. CH),7.46-7.48 (2H, d, Ar. CH), 7.69-7.70 ppm (2H, d, Ar. CH); MS (ESI) m/z [% rel. abundance] : 320 (100) (M^+), 321 (23) $[M+1]^+$, 244(68), 131(42), 117(28), 91(83); elemental analysis: calcd for $C_{20}H_{24}N_4$: C, 74.97; H, 7.55; N, 17.48. found: C, 74.94; H, 7.52; N, 17.47 %.

2-(4-Methylphenyl-1-(ethylpiperazin-1-yl) methyl)-*1H*-benzo*[d]*imidazole (2n):

UV λ_{max} (DMSO): 272.0 nm; IR (KBr)ν:3080 (aromatic C-H stretching),2958 (aliphatic C-H stretching), 1685 (aromatic C=N stretching), 1602 (aromatic C-C stretching), 1554 (aromatic C=C stretching), 1292(aromatic C-N stretching), 1105 (aliphatic C-N stretching), 847 cm^{-1} (C-H (*p*-disubstituted benzene) (def.)); ^{1}H NMR (DMSO-d_6): δ1.15-1.17 (3H, t, CH_2), 2.32-2.34 (2H, m, N-CH_2), 2.40-2.41 (4H, t, N-CH_2), 2.45-2.48 (4H, t, CH_2), 2.52 (3H, s, CH_3), 4.81 (2H, s, N-CH_2), 7.11-7.12 (2H, d, Ar. CH), 7.31-7.32 (2H, t, Ar. CH), 7.33-7.34 (1H, d, Ar. CH), 7.36-7.38 (1H, d, Ar. CH),7.71-7.72 ppm (2H, d, Ar. CH); MS (ESI) m/z [% rel. abundance] : 334 (100) (M^+), 335 (24) $[M+1]^+$, 320(81), 244(28), 131(37), 117(72), 91(55); elemental analysis: calcd for $C_{21}H_{26}N_4$: C, 75.41; H, 7.84; N, 16.75; found: C, 75.39; H, 7.80; N, 16.72 %.

Anthelmintic activity

Anthelmintic activity was evaluated on earthworm, *Phaeritima posthuma*. Earthworms were divided into fourteen groups (5 each). The first group served as normal control which received Tween®80 (0.5% w/V) and distilled water only. The second received the standard drug i.e. mebendazole in Tween®80 (0.5% w/V) and distilled water at a dose level of 100 mg as per 'IP' and remaining test groups received different concentration of 1, 2, and 4mg/mL doses of synthesized compounds in Tween®80 (0.5% w/V)and distilled water. The triplicate study was performed. Observations were made for the time taken to cause paralysis and death of individual worms for two hour. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworm in warm water (50°C), which stimulate the movement, if the worm was alive. The results of anthelmintic activity are shown inTable II.

Antibacterial activity

The antibacterial activity of newly synthesized compounds was tested by paper disc diffusion method using nutrient agar medium against the following bacterial strains: *S. aureus* (NCIM 2079), *P. aeureginosa* (ATCC 10145), *B. subtilis* (NCIM 2063) and *E. coli* (ATCC 11775).

In the paper disc-diffusion method, paper disc impregnated with test compounds dissolved in DMSO at concentration 25, 50 and 100 µg/mL were used. Disc impregnated with DMSO solvent were used as control for antibacterial activity because of free solubility of test compounds. Ciprofloxacin at the concentration of 50µg mL^{-1} was used as standard drug for antibacterial activity. The microorganism culture was spread over nutrient agar media in petri dishes, and then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 hrs for bacterial cultures. After incubation, the zones of inhibition around the disc were observed. The zones of inhibition indicate that the compounds

inhibit growth of microorganism. Each testing was done in triplicate. Results were interpreted in terms of diameter (mm) of zone of inhibition.The results of antibacterial studies are presented in Table III.

RESULTS AND DISCUSSION

The structures of the synthesized compounds were characterized on the basis of FTIR, ^{1}H NMR, EIMS spectral data and elemental analysis. Physical data like m.p., R_f value and percentage yield of all the synthesized compounds, are shown in Table I.

The newly synthesized compounds were screened for anthelmintic and antibacterial activities. The anthelmintic activity was performed on *Phaeritima posthuma* species of earthworm, compared to standard drug mebendazole. The compound 2a, 2b, 2e and 2h showed potent anthelmintic activity.

The antibacterial activity was screened against Gram positive and Gram negative bacterial strains. The compound 2e, 2h and 2k showed significant antibacterial activity against gram positive bacterial strain like *B. subtilis* (NCIM 2063) and *S. aureus* (NCIM 2079) and compound 2l and 2m showed significant antibacterial activity against gram negative bacterial strains like *E.coli* (ATCC 11775) and *P. aeruginosa* (ATCC 10145).

The results of anthelmintic and antibacterial studies indicate that significant activity of the newly synthesized benzimidazole derivatives was found in derivatives with piperazine and *N*-methyl piperazine in combination with *p*-chloro and *o*-nitro benzoic acid.

ACKNOWLEDGEMENT

The authors are grateful to the management of Rajiv Academy for Pharmacy, Mathura (U.P.) for providing facilities for this work. The authors are also thankful to Central Drug Research Institute (CDRI), Lucknow for providing elemental analysis and spectral data of the synthesized compounds.

REFERENCES

1. Venkataramana H.S., Singh A., Tiwari A. and Tiwari V.:Synthesis of phenyl hydrazine substituted benzimidazole derivatives and their biological activity, **Int. J. Pharm. Sci. Res.** 2010, 1(1), 34-38.
2. Gaba M., Singh D., Singh S., Sharma V. and Gaba P.: Synthesis and pharmacological evaluation of novel 5-substituted-1-(phenylsulfonyl)-2-methylbenzimidazole derivatives as anti-inflammatory and analgesic agents, **Eur. J. Med. Chem.** 2010, 45, 2245-2249.
3. Pathak D., Siddiqui N., Bhrigu B., Ahsan W. and Alam M.S.: Benzimidazoles: a new profile of biological activities, **Der Pharmacia Lett.** 2010, 2(2), 27-34.
4. Sharma S., Gangal S., and Rauf A.: Convenient one-pot synthesis of novel 2-substituted benzimidazoles, tetrahydrobenzimidazole and imidazole and evaluation of their *in vitro* antibacterial and antifungal activities, **Eur. J. Med. Chem.** 2009, 44, 1751-1757.
5. Zhang D., Wang Z., Lida-Tang F. and Wang J.: Design, synthesis and antibacterial activity of novel actinonin derivatives containing benzimidazole heterocycles. **Eur. J. Med. Chem.,** 2009, 44, 2202-2210.
6. He Y., Wu B., Yang J., Robinson D., Risen L., Ranken R., Sheng S. and Swayze E.: 2-Piperidin-4-yl-benzimidazoles with broad spectrum antibacterial activities, **Bioorg. Med. Chem. Lett.** 2003, 13, 3253-3256.
7. He Y., Yang J., Risen L. and Swayze E.: Synthesis and biological evaluations of novel benzimidazoles as potential antibacterial agents, **Bioorg. Med. Chem. Lett.** 2007, 14, 1217-1220.
8. Goker H., Ozden S., Yildiz S. and Boykin D.: Synthesis and potent antibacterial activity against MRSA of some novel benzimidazole-*N*-alkylated-5-carboxamidines, **Eur. J. Med. Chem.** 2005, 40, 1062-1067.
9. Guven O.O., Goker H. and Yildiz S.: Synthesis and antimicrobial activity of some novel phenyl and benzimidazole substituted benzyl ethers, **Bioorg. Med. Chem.** 2007, 17, 2233-2236.
10. Kumar D., Jacob M.R., Reynolds M.B. and Kerwin S.M.: Synthesis and evaluation of anticancer benzoxazoles and benzimidazoles related to UK-1, **Bioorg. Med. Chem.** 2002, 10, 3997-4004.
11. Rida S.M., El-Hawash S.A.M., Fahmy H.T.Y., Hazzaa A.A. and El-Meligy M.M.: Synthesis of novel benzofuran and related benzimidazole derivatives for evaluation of *in vitro* anti-HIV-1, anticancer and antimicrobial activities, **Arch. Pharm. Res.** 2006, 29(10), 826-833.
12. Vinod Kumar R., Vaidya S.D., Sivakumar B.V., Bhise U.N., Bhirud S.B. and Mashelkar U.C.:Synthesis, anti-bacterial, anti-asthmatic and anti-diabetic activities of novel N-substituted-2-(4-phenylethynyl-phenyl)-1*H*-benzimidazoles and N-substituted 2-[4-(4,4-dimethyl thiochroman-6-yl-ethynyl)-phenyl)-1*H*-benzimidazoles, **Eur. J. Med. Chem.** 2008, 43, 986-995.
13. Sreena K., Ratheesh R., Rachana M., Poornima M.and Shyni C.:Synthesis and anthelmintic activity of benzimidazole derivatives, **HYGEIA.** 2009, 1(1), 21-22.

14. Gaba M., Singh D., Singh S., Sharma V. and Gaba P.: Synthesis and pharmacological evaluation of novel 5-substituted-1-(phenylsulfonyl)-2-methylbenzimidazole derivatives as anti-inflammatory and analgesic agents, **Eur. J. Med. Chem.** 2010, 45, 2245-2249.
15. Sondhi S.M., Singh N., Kumar A., Lozach O. and Meijer L.:Synthesis, anti-inflammatory, analgesic and kinase (CDK-1, CDK-5 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases, **Bioorg. Med. Chem. Lett.** 2006, 14, 3758-3765.
16. Kerimov I., Ayhan-Kilcigil G., Benay Can-Eke B., Altanlar N. and Iscan M.: Synthesis, antifungal and antioxidant screening of some novel benzimidazole derivatives, **J. Enzym. Inhib. Med. Chem.** 2007, 22(6), 696-701.
17. Gupta S.K., Pancholi S.S., Gupta M.K., Agrawal D. and Khinchi M.P.: Synthesis and biological evaluation of some 2-substituted derivatives of benzimidazoles, **J. Pharm. Sci. Res.** 2010, 2(4), 228-231.
18. Dahiya R. and Pathak D.: Synthetic studies on novel benzimidazolopeptides with antimicrobial, cytotoxic and anthelmintic potential, **Eur. J. Med. Chem.** 2007, 42, 772-798.
19. Sharma G.K. and Pathak D.: Microwave-assisted synthesis of some imidazoles of biological interest, **Indian J. Heterocycl. Chem.** 2009, 19, 203-204.
20. Sharma G.K. and Pathak D.: Microwave-assisted, solvent-free and parallel synthesis of some novel substituted imidazoles of biological interest, **Chem. Pharm. Bull.** 2010, 58, 375-380.
21. Sharma G.K. and Pathak D.: 3D-QSAR study on ring substituted imidazoles for their antitubercular activity, **Letters in Drug Design and Discovery** 2010, 7, 128-132.
22. Siddiqui N., Bhrigu B., Pathak D., Alam S. and Ali R.: Cytotoxicity and enzymes estimation of some newer benzimidazoles, **Annals of Biological Res.** 2011, 2, 194-199.
23. Khalafi-Nezhad A., Soltani M.N., Mohabatkar H. and Hemmateenejad B.: Design, synthesis, antibacterial and QSAR studies of benzimidazole and imidazole's chloroaryloxyalkyl derivatives. **Bioorg. Med. Chem.** 2005, 13, 1931-1938.