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RESEARCH ARTICLE

Synthesis and Characterizaton of Some *N*-Mannich Bases as Potential Antimicrobial, Anthelmintic and Insecticidal Agents

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Abstract: A series of *N*-Mannich bases of benzimidazolyl substituted 1*H*-isoindole-1,3(2H) dione have been derived by the reaction of different substituted amino acids with phthalic anhydride to yield 2-(substituted) 1*H*-isoindole-1,3(2*H*) diones (**3**), further condensation with *o*-phenylenediamine yields 2-substituted benzimidazolyl 1*H*-isoindole-1,3(2H) diones (**4**), followed mannich reaction with methanal and different amines to yields final products (**5**). The chemical structures of synthesized *N*-Mannich bases were determined by elemental analysis and spectral data (FTIR & ^{1}H NMR). All the synthesized derivatives have been evaluated for their antimicrobial, anthelmintic and insecticidal activities against microbes, helminthes and insects selected as compared to standard drugs by using disc diffusion method and Watkins technique respectively. All the synthesized *N*-Mannich bases possess the significant antimicrobial, anthelmintic and insecticidal activities.

Keywords: Mannich base, 1*H*-isoindole-1, 3(2*H*)-dione, Antimicrobial, Anthelmintic, Insecticidal activities

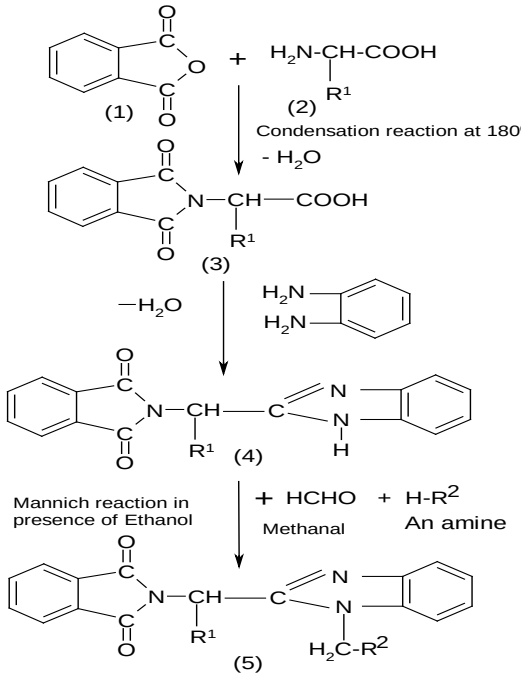
Introduction

Isoindole -1, 3(2*H*) – dione[1] is an aromatic imide, contains isoindole moiety, which is motif in nature. In the combined form with maleimides and succinimides, isoindole -1,3(2*H*)-diones used as plastic modifiers to improve heat resistance, antioxidant and anti-foulant properties. Isoindole -1,3(2*H*) – dione and its derivatives have received much attention owing to the varied biological and pharmaceutical activities including antimicrobial[2,3], antihypertensive[4,5], anti-viral[6], antitumer[7] anti-inflammatory agents[8,9], as a inhibitors of HIV-I integrase[10] and serve as ligands to form bioactive metal complexes. They also exhibit liquid phase crystalline properties[11] with antibacterial activities.

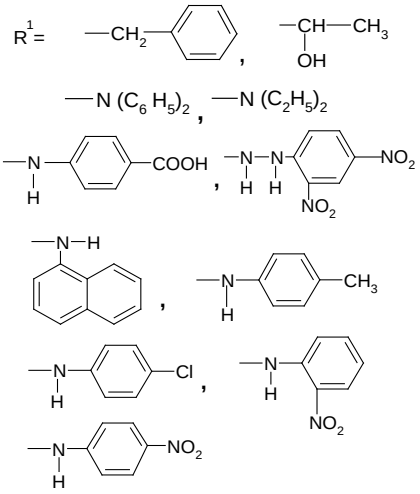
In the present communication we have synthesized *N*-Mannich bases of benzimidazolyl substituted 1*H*-isoindole-1, 3(2H)dione (Scheme1), characterized and screened for their antimicrobial, anthelmintic and insecticidal activities.

Experimental

The melting points were recorded by open capillary method and are uncorrected. IR spectra (λ_{max} in cm^{-1}) were recorded on a Shimadzu FTIR 8300 spectrophotometer using KBr pellets. The ^1H NMR spectra were recorded on a DRX-300 (300 MHz) instrument using $CDCl_3$ as solvent (chemical shift in δ ppm) and TMS as internal standard. The completion of reactions was monitored by TLC.



Where:



Scheme 1

Experimental

10 g of Phthalic anhydride and 5.0 g of different amino acids (one by one) were taken in a conical flask (Borosil) and immersed in a previously heated oil bath (170^0-180 ^{0}C). The mixture was stirred occasionally during the first 10 minutes and any phthalic anhydride which sublimed, was pushed down into the reaction mixture, till there was complete fusion. The mixture was kept undisturbed for 5 minutes, when the liquid mass solidified. The solid obtained was then recrystallized from 10% ethanol. The other derivatives were prepared with the same procedure using different amino acids.

*Step-2: Synthesis of 2-substituted benzimidazolyl-1H-isoindole-1, 3 (2H)-diones (**4a-l**)*

0.1 Mol of different compounds of step-1 (one by one) and 0.1 mol of *o*-phenylene diamine were refluxed in 30 mL.of 4N HCl for 2 hours. The solution on cooling gave a precipitate which was filtered, dried and recrystallized from ethanol. The purity of compounds has been checked by TLC using silica gel-G (activated). The other derivatives were prepared with the different compound of step-1(**3a-l**), applying the same procedure.

Yield 70-75%, m.p. 200-210 ^{0}C, m.wt. 350-450, IR(KBr) in cm^{-1}: 3090-3110(Ar-H str.), 1605-1620(C=C str.), 1581-1589(C=N str.), 2850-2875(C-H str.), 1770-1779 & 1710-1720(C=O str. in phthalimide ring), 740-745 (Ar-H def. monosubsti. ring), 3330-3351 (N-H str. amine sec.), 3000-3399(O-H str. alcoholic–OH); ^{1}HNMR ($CDCl_3$) δ(ppm): 5.22-5.26 (τ, 1H, CH-CH_2), 3.72-3.75(δ, 1H, -O<u>H</u>), 4.70-4.75(δ, 1H, N-<u>H</u>), 7.10-8.10(m, 8H, Ar-H & 13H, Ar-H).

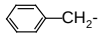
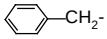
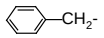
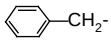
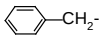
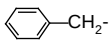
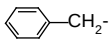
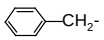
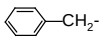
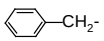
*Step-3(a): Synthesis of Mannich bases of 2-substituted-(1H-aminomethyl substituted-benzimidazolyl)-1H-isoindole-1,3(2H) diones(**5 a-i**)*

0.02 Mol of different compounds of step-2 (one by one) was dissolved in 4 mL of 35% methanal in ethanol. To this was added 0.04 mol of different amines (gradually with stirring) and then stirred it for 1 hour at room temperature. The mixture was left overnight. The solid which was separated out, was filtered, dried and recrystallized from ethanol. The purity of compounds has been checked by TLC. The other derivatives of substituted isoindole-1,3-(2H)-diones were prepared with different amines (pri. & sec.) and methanal, applying the same procedure.

*Step-3(b): Synthesis of Mannich bases of 2-substituted-(1H-aminomethyl substituted-benzimidazolyl)-1H-isoindole-1, 3 (2H) diones (**5j –l**)*

0.02 Mol of different compounds of step-2 (one by one), was dissolved in 4 mL of 35% methanal in ethanol. To this was added 0.04 mol of different anilines (2-nitro and 4-nitro-anilines) and the mixture was refluxed for 4-5 hours on a water bath. The solution was left overnight in a freezer. The solid obtained was filtered, dried and recrystallised from ethanol. The purity of compounds has been checked by TLC. The other derivatives of substituted-1, 3-(2*H*)-diones were prepared with different anilines and methanal applying the same procedure. The yields, melting points, elemental analysis results of synthesized heterocyclic derivatives have been given in the following table:

Table 1. The Physical data of synthesized derivatives **5a-l**

S. No.	Comp. code	R^1	R^2	M.F.	M.wt.	m.p., °C	Yield, %	Elemental Analysis %			
									C	H	N
1.	5a		Piperazino-	$C_{28}H_{27}N_5O_2$	465.0	138	58	found	72.21	5.78	14.99
								calc.	72.25	5.80	15.05
2.	5b		Morpholino-	$C_{28}H_{26}N_4O_3$	466.0	140	70	found	72.08	5.52	10.28
								calc.	72.10	5.57	10.30
3.	5c		Diphenyl-amino-	$C_{36}H_{28}N_4O_2$	548.0	160	82	found	78.79	5.09	10.20
								calc.	78.83	5.10	10.21
4.	5d		Diethylamino-	$C_{28}H_{28}N_4O_2$	452.0	162	70	found	74.30	6.17	12.35
								calc.	74.33	6.19	12.38
5.	5e		4-Carboxy-phenylamino-	$C_{31}H_{24}N_4O_2$	516.0	274	88	found	72.05	4.62	10.82
								calc.	72.09	4.65	10.85
6.	5f	CH_3-CH(OH)-	Diphenyl-amino-	$C_{31}H_{26}N_4O_3$	502.0	122	65	found	74.08	5.14	11.12
								calc.	74.10	5.17	11.15
7.	5g		1*H*-α-naphthylamino-	$C_{34}H_{26}N_4O_2$	522.0	163	88	found	78.14	4.96	10.70
								calc.	78.16	4.98	10.72
8.	5h		4-Tolylamino-	$C_{31}H_{26}N_4O_2$	486.0	101	58	found	76.51	5.31	11.50
								calc.	76.54	5.34	11.52
9.	5i		4-Chloro-phenylamino-	$C_{30}H_{23}N_4O_2$-Cl	506.0	180	71	found	71.03	4.51	11.02
								calc.	71.07	4.54	11.05
10.	5j		2-Nitro-phenylamino-	$C_{30}H_{23}N_5O_4$	517.0	225	86	found	69.61	4.42	13.50
								calc.	69.63	4.44	13.53
11.	5k		4-Nitro-phenylamino-	$C_{30}H_{23}N_5O_4$	517.0	199	88	found	69.60	4.41	13.50
								calc.	69.63	4.44	13.53
12.	5l		2,4-Dinitro-phenyl hydrazino-	$C_{30}H_{23}N_7O_6$	577.0	150	85	found	62.37	3.96	16.95
								calc.	62.39	3.98	16.98

Spectral data[12,13] **of derivatives 5(a-l)**

*2-Phenylethyl-(1H-piperazinomethyl-benzimidazolyl)-1H-isoindole-1,3(2H)-dione (**5a**)*

IR (KBr, cm^{-1}): 3055.20(Ar-H str.), 1678.30(C=C str.), 745.00(Ar-H def. mono. subst. ring), 1772.64&1716.70 (C=O str. phthalimide ring), 1410.11(C-N str. cyclic),1595.40 (C=N str. benzimidazole ring), 1335.60(N-C str. aromatic amine tert.),1108.20(N-C str. aliphatic tert. amine), 2864.30 (C-H str. ring substi.in CH_2-), 3310.50(N-H str. sec.); ^{1}H NMR ($CDCl_3$) δ(ppm) : 3.06-3.30(d-d, 2H, CH-C$\underline{H}_2$), 4.70(δ, 1H, N-$\underline{H}$), 5.24(τ,1H, C$\underline{H}$-CH_2), 5.60 (δ, 2H, N-C$\underline{H}_2$-N), 2.32-2.64 (τ, 4H, C$\underline{H}_2$-N-C$\underline{H}_2$), 7.12-8.00(m, 13H, Ar-$\underline{H}$).

*2-Phenylethyl-(1H-morpholinomethyl-benzimidazolyl)-1H-isoindole-1,3(2H)-dione (**5b**)*

IR (KBr, cm^{-1}): 3057.27(Ar-H str.), 1680.34(C=C str.), 746.46(Ar-H def. mono.subst.ring), 1770.71&1714.70 (C=O str. phthalimide ring), 1410.13(C-N str. cyclic),1595.18 (C=N str. benzimidazole ring), 1338.64(N-C str. aromatic amine tert.),1105.25(N-C str. aliphatic tert. amine), 2863.39 (C-H str. ring substi.in CH_2-), 1257.63(C-O-O str.), 949.01(C-O-O bending); ^{1}HNMR ($CDCl_3$) δ(ppm): 3.04-3.29(d-d, 2H , CH-C$\underline{H}_2$), 5.22(τ,1H, C$\underline{H}$-CH_2), 5.59 (δ, 2H, N-C$\underline{H}_2$-N), 2.31-2.61(τ, 4H, C$\underline{H}_2$-N-C$\underline{H}_2$), 3.45-3.70(τ, 4H, C$\underline{H}_2$-O-C$\underline{H}_2$ in morpholine ring), 7.16-8.02 (m, 13H, Ar-$\underline{H}$).

*2-Phenylethyl-(1H-diphenylaminomethyl-benzimidazolyl)-1H-isoindole-1,3(2H)-dione (**5c**)*

IR (KBr, cm^{-1}): 3030.28(Ar-H str.), 1655.34(C=C str.), 750.33(Ar-H def. mono.subst.ring), 1772.61&1716.70 (C=O str. phthalimide ring), 1404.30(C-N str. cyclic),1573.97(C=N str. benzimidazole ring), 1337.64(N-C str. aromatic amine tert.),1106.22(N-C str. aliphatic tert. amine), 2870.17 (C-H str. ring substi.in CH_2-); ^{1}H NMR ($CDCl_3$) δ(ppm) : 3.05-3.31 (d-d, 2H, CH-C$\underline{H}_2$), 5.23(τ,1H, C$\underline{H}$-CH_2), 5.58 (δ, 2H, N-C$\underline{H}_2$-N), 7.15-8.05(m, 23H, Ar-$\underline{H}$).

*2-Phenylethyl-(1H-diethylaminomethyl-benzimidazolyl)-1H-isoindole -1,3 (2H)-dione (**5d**)*

IR (KBr, cm^{-1}): 3052.28(Ar-H str.), 1602.34(C=C str.), 746.48(Ar-H def. mono.subst.ring), 1772.61&1716.70 (C=O str. phthalimide ring), 1415.30(C-N str. cyclic),1573.98(C=N str. benzimidazole ring), 1350.22(N-C str. aromatic amine tert.),1103.25(N-C str. aliphatic tert. amine), 2875.96 (C-H str. ring substi.in CH_2-), 2978.19(C-H str. in –CH_3); ^{1}H NMR ($CDCl_3$) δ(ppm) : 1.06(τ, 6H(β), N-(CH_2-C$\underline{H}_3$)$_2$, 2.65(q, 4H(α), N-(C$\underline{H}_2$-CH_3)$_2$, 3.02-3.29 (d-d, 2H , CH-C$\underline{H}_2$), 5.20(τ,1H, C$\underline{H}$-CH_2), 5.54 (δ, 2H, N-C$\underline{H}_2$-N), 7.16-8.15(m, 13H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H-(4-carboxyphenyl amino)methyl- benzimidazolyl]-1H-isoindole -1,3 (2H)-dione (**5e**)*

IR (KBr, cm^{-1}): 3028.34(Ar-H str.), 1605.94(C=C str.), 769.33(Ar-H def. mono.subst.ring), 837.13(Ar-H def. *p*-disubsti. benzene ring),1772.63&1716.60 (C=O str. phthalimide ring), 1421.58(N-C str. cyclic),1574.97(C=N str. benzimidazole ring), 1330.97(N-C str. aromatic amine tert.), 2897.18 (C-H str. ring substi.in CH_2-), 3360.31(N-H str. aliphatic amine sec.), 1680.04(C=O str. in –COOH), 3300.05(O-H str.), 1390.65(C-O-H bending); ^{1}H NMR ($CDCl_3$) δ(ppm) : 3.05-3.32(d-d, 2H, CH-C$\underline{H}_2$), 4.78(δ, 1H, N-$\underline{H}$), 5.26(τ,1H, C$\underline{H}$-CH_2), 5.61 (δ, 2H, N-C$\underline{H}_2$-N), 7.15-8.02(m, 17H, Ar-$\underline{H}$), 10.91(δ, 1H, -COO$\underline{H}$).

*2-[(2'-Hydroxypropyl)-1H-(diphenylamino-methyl-benzimidazolyl)]-1H-isoindole-1,3 (2H)-dione (**5f**)*

IR (KBr, cm^{-1}): 3028.35(Ar-H str.), 1614.47(C=C str.), 746.55(Ar-H def. mono.substi. benzene ring), 1776.70 &1700.09(C=O str. phthalimide ring), 1410.01(N-C str. cyclic), 1544.12(C=N str. benzimidazole ring), 1311.65(N-C str. aromatic amine tert.), 2879.82(C-H str. ring substi.in CH_2-), 2918.40(C-H str. in –CH_3), 3360.66(O-H str.in alcoholic-OH); ^{1}H NMR ($CDCl_3$) δ(ppm): 1.18-1.29(d, 3H, -CH(OH)-C$\underline{H}_3$), 3.75(δ, 1H, -O$\underline{H}$), 4.32-4.50 (q, 1H, -C$\underline{H}$(OH)-CH_3), 4.84-4.99(d, 1H, -C$\underline{H}$-CH(OH)-CH_3), 5.51(δ, 2H, N-C$\underline{H}_2$-N), 7.16-8.02(m, 18H, Ar-$\underline{H}$).

*2-Phenylethyl-[(1H-α-naphthyl) amino-methyl-benzimidazolyl]-1H-isoindole-1,3 (2H)-dione (**5g**)*

IR (KBr, cm^{-1}): 3028.44(Ar-H str.), 1600.97(C=C str.), 721.40(Ar-H def. mono.substi. ring), 873.35(Ar-H def.1,2,3-trisubsti. benzene ring),1774.57&1714.78(C=O str. phthalimide ring), 1388.79(N-C str. cyclic), 1579.97(C=N str. benzimidazole ring), 1339.91(N-C str. aromatic amine tert.), 2873.11(C-H str. ring substi.in CH_2-), 3350.13(N-H str.); ^{1}H NMR ($CDCl_3$) δ(ppm) : 3.04-3.32(d-d, 2H, CH-C$\underline{H}_2$), 4.73(δ, 1H, Ar-N-$\underline{H}$), 5.23(τ,1H, C$\underline{H}$-CH_2), 5.60 (δ, 2H, N-C$\underline{H}_2$-N), 7.08-8.06(m, 20H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H-(4-tolylamino)-methyl benzimidazolyl]-1H-isoindole-1,3 (2H)-dione (**5h**)*

IR (KBr, cm^{-1}): 3038.44(Ar-H str.), 1611.20(C=C str.), 735.12(Ar-H def. mono.substi. ring), 840.15(Ar-H def. *p*-disubsti. benzene ring), 1775.61&1712.77(C=O str. phthalimide ring), 1420.87(N-C str. cyclic), 1578.85(C=N str. benzimidazole ring), 1330.71(N-C str. aromatic amine tert.), 2870.22(C-H str. ring substi.in CH_2-), 3351.10(N-H str. aromatic amine sec.), 2980.10(C-H str. in CH_3); ^{1}H NMR ($CDCl_3$) δ(ppm) : 1.08(δ, 3H, -C$\underline{H}_3$), 3.02-3.29(d-d, 2H , CH-C$\underline{H}_2$), 4.72(δ, 1H, Ar-N-$\underline{H}$), 5.21(τ,1H, C$\underline{H}$-CH_2), 5.18(δ, 2H, N-C$\underline{H}_2$-N), 7.10-8.01 (m, 17H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H-(4-chlorophenyl amino)-methyl-benzimidazolyl]-1H-isoindole-1,3 (2H)-dione (**5i**)*

IR (KBr, cm^{-1}): 3062.11(Ar-H str.), 1631.12(C=C str.), 750.22(Ar-Cl str. aromatic ring substitution), 768.01(Ar-H def. mono.substi.ring), 835.18(Ar-H def. *p*-disubsti. benzene ring), 1773.11&1714.80(C=O str. phthalimide ring), 1420.55(N-C str. cyclic), 1545.81(C=N str. benzimidazole ring), 1330.94(N-C str. aromatic amine tert.), 2890.22(C-H str. ring substi.in CH_2-), 3362.51(N-H str. aromatic amine sec.); ^{1}H NMR ($CDCl_3$) δ(ppm) : 3.06-3.32(d-d, 2H, CH-C$\underline{H}_2$), 4.75(δ, 1H, Ar-N-$\underline{H}$), 5.24(τ,1H, C$\underline{H}$-CH_2), 5.58(δ, 2H, N-C$\underline{H}_2$-N), 7.11-8.05(m, 17H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H-(2-nitrophenyl amino)-methyl-benzimidazolyl]-1H-isoindole-1,3 (2H)-dione (**5j**)*

IR (KBr, cm^{-1}): 3067.15(Ar-H str.), 1610.22(C=C str.), 761.55(Ar-H def. mono.substi. benzene ring), 842.72(Ar-H def. o-disubsti. benzene ring), 1771.68 &1715.20(C=O str. phthalimide ring), 1422.00(N-C str. cyclic), 1565.11(C=N str. benzimidazole ring), 1328.99(N-C str. aromatic amine tert.), 2889.10(C-H str. ring substi.in CH_2-), 3339.51(N-H str. aromatic amine sec.), 1521.90(N=O str. asym), 1312.15(N=O str. sym); ^{1}H NMR ($CDCl_3$)δ(ppm) : 3.01-3.30(d-d, 2H, CH-C$\underline{H}_2$), 4.77(δ, 1H, Ar-N-$\underline{H}$), 5.21(τ,1H, C$\underline{H}$-CH_2), 5.66(δ, 2H, N-C$\underline{H}_2$-N), 7.13-8.10(m, 17H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H(4-nitrophenyl amino)-methyl- benzimidazolyl]-1H-isoindole-1,3 (2H)-dione (**5k**)*

IR (KBr, cm^{-1}): 3060.11(Ar-H str.), 1615.01(C=C str.), 758.11(Ar-H def. mono.substi. benzene ring), 838.90(Ar-H def. p-disubsti. benzene ring), 1774.00 &1712.28(C=O str. phthalimide ring), 1419.11(N-C str. cyclic), 1540.15(C=N str. benzimidazole ring), 1330.05(N-C str. aromatic amine tert.), 2869.12(C-H str. ring substi.in CH_2-), 3341.11(N-H str. aromatic amine sec.), 1512.19(N=O str. asym), 1300.09(N=O str. sym); ^{1}H NMR ($CDCl_3$)δ(ppm) : 3.03-3.34(d-d, 2H, CH-C$\underline{H}_2$), 4.76(δ, 1H, Ar-N-$\underline{H}$), 5.23(τ,1H, C$\underline{H}$-CH_2), 5.61(δ, 2H, N-C$\underline{H}_2$-N), 7.15-8.12(m, 17H, Ar-$\underline{H}$).

*2-Phenylethyl-[1H-(2,4-dinitrophenylhydrazino)-methyl-benzimidazolyl]-1H-isoindole-1,3 (2H)-dione(**5l**)*

IR (KBr, cm^{-1}): 3091.99(Ar-H str.), 1610.88(C=C str.), 744.55(Ar-H def. mono.subst.ring), 831.35 (Ar-H def.1,2,3-trisubsti. benzene ring),1771.64&1711.68(C=O str. phthalimide ring), 1420.00(N-C str. cyclic), 1589.40(C=N str. benzimidazole ring), 1336.91(N-C str. aromatic amine tert.), 2872.10 (C-H str. ring substi. in CH_2-), 15.19.96(N=O str. asym.), 1311.64(N=O str. sym.), 3309.96(N-H str.); ^{1}H NMR ($CDCl_3$) δ(ppm) : 2.60(δ, 1H, CH_2-N-H hydrazino grp.), 3.05-3.31(d-d, 2H, CH-CH_2), 4.81(δ, 1H, Ar-N-H), 5.26(τ,1H, CH-CH_2), 5.59 (δ, 2H, N-CH_2-N), 7.19-8.05(m, 16H, Ar-H).

Antimicrobial activity[14,15]

The synthesized derivatives (**5a-l**) were screened for their *in vitro* antimicrobial activity against, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and *antifungal activity against, Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride & Candida albicans* by measuring the zone of inhibition in mm. The antimicrobial activity was performed by standard filter paper disc diffusion method and zone of inhibition, reported in the Table 2, using 100 µg/mL concentrations of synthesized isoindole-1, 3 (2*H*)-dione derivatives. Streptomycin and nystatin were used as standard drug for antibacterial and antifungal activities respectively. Nutrient agar was employed as culture medium and DMSO were used as solvent.

Table 2. Antimicrobial data of derivatives **5a-l**

S. No.	Compounds code	Antibacterial				Antifungal			
		B. Subtilis	*E. coli*	*K. pneumoniae*	*. S. aureus*	*A. niger*	*A. flavous*	*T. viride*	*C. albicans*
1.	5a	++	+++	++	++	++	+++	+++	+++
2.	5b	+++	+++	+++	+++	++	++	+	++
3.	5c	+++	++	++	+++	++	+	++	-
4.	5d	++	+	+	++	++	+++	+++	++
5.	5e	+++	++++	+++	++++	++	++	++	++
6.	5f	+++	++	++	-	+	+	++	+++
7.	5g	+++	+++	++	+++	+++	++	+++	+++
8.	5h	++	++	++	++	+++	++	++	+++
9.	5i	+++	+++	++	+++	++	+++	+++	+++
10.	5j	++	+	+	+	+	++	+++	++
11.	5k	+++	++	++	++	++	+++	++	+++
12	5l	+++	+++	++	+++	++	++	++	+++
Std. drug	Streptomycin	++++	+++	+++	++++	-	-	-	-
	Nystatin	-	-	-	-	++++	+++	++++	+++

Zone of inhibition was measured in mm, ++++: (18-20 mm) Strong activity, +++ :(15-18 mm) good activity, ++: (10-15 mm) moderate activity, + : (8-10)slight active, - : (<8)inactive

In vitro anthelmintic activity[16]

In vitro anthelmintic screening studies of synthesized isoindole-1, 3 (2*H*)-dione derivatives **5a-l** were performed by the watkins technique**,** against common Indian earthworm *'P. posthuma"*. For this purpose 4% and 2% solutions of the synthesized derivatives and standard drug piperazine hydrochloride in ethylene glycol were used for experiment. The experiments were performed in duplicate and average values of paralytic time and lethal time in minutes have given in the Table 3.

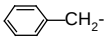
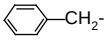
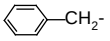
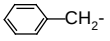
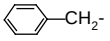
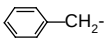
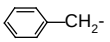
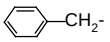
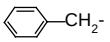
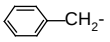
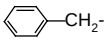
Table 3. Results of anthelmintic activity of synthesized derivatives **5a-l**

S. No	Compounds code	Concentration			
		4%		2%	
		Mean paralytic time, min.	Mean lethal time, min.	Mean paralytic time, min.	Mean lethal time, min.
1	5a	7	13	8	15
2	5b	8	13	7	14
3	5c	8	14	8	16
4	5d	9	15	11	17
5	5e	8	15	9	16
6	5f	6	13	9	17
7	5g	10	17	10	17
8	5h	11	17	12	18
9	5i	8	14	10	17
10	5j	9	15	10	16
11	5k	8	15	8	16
12	5l	9	15	8	17
13	Std. drug	6	13	7	15

Insecticidal activity[17,18]

Adult cockroaches (*P. americana)* were selected for the testing of *in vitro* insecticidal activity. 2% Solution of synthesized isoindole-1, 3 (2*H*)-dione derivatives and standard drug cypermethrin (w/v) in acetone were used for experiment. The time of death of cockroaches was noted as KD (Knock Down) value in minutes. For each sample three replication were performed and same experiments were performed with the standard drug.

Table 4. Results of insecticidal activity of synthesized derivatives **5a-l**

S. No.	Compounds code	R^1	R^2	Mean K.D. Value (Min)
1	5a		Piperazino-	9
2	5b		Morpholino-	8
3	5c		Diphenylamino-	10
4	5d		Diethylamino-	10
5	5e		4-Carboxyphenyl-amino-	9
6	5f	CH_3-CH(OH)-	Diphenylamino-	9
7	5g		1*H*-α-naphthylamino-	10
8	5h		4-Tolylamino-	11
9	5i		4-Chlorophenyl- amino-	8
10	5j		2-Nitrophenyl- amino-	8
11	5k		4-Nitrophenyl- amino-	9
12	5l		2,4- Dinitrophenyl-hydrazino-	8
13	Std.drug	Cypermethrin		8

Conclusion

All the synthesized *N*-Mannich bases of isoindole-1, 3 (2*H*)-dione derivatives **5a-l** have given appreciable yield with satisfactory elemental analysis and spectral data. It is inferred from the Table 2, 3 and 4, that synthesized derivatives **5a-l** have exhibited significant anthelmintic insecticidal and antibacterial activities, while moderate to good antifungal activity against helminthes, insect, bacteria & fungus selected, as compared to the standard drugs. Due to increase of basic moiety, derivatives associated with piperazino, morpholino, diphenylamino, nitro & chloro groups produced better activity than the rest. On passing toxicity tests these derivatives may prove to be a good antibacterial agent and potent insecticidal & anthelmintic agents of future.

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