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Original article

Synthesis and antibacterial screening of hydrazones, Schiff and Mannich bases of isatin derivatives

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Abstract – Schiff bases and hydrazones of substituted isatins (**1**–**28**) were prepared by reacting isatin and aromatic primary amines/hydrazines. A new series of the corresponding *N*-Mannich base (**29**–**35**) was synthesised by reacting them with formaldehyde and diphenyl amine. The chemical structures were confirmed by means of ^{1}H-NMR, IR spectral data and elemental analysis. The compounds were screened for antibacterial activity against seven Gram (+) and seven Gram (−) standard and pathological bacterial strains by the paper disc diffusion technique. The minimum inhibitory concentrations of the active compounds were determined. 1-Diphenyl amino-methyl-3-(4-bromo phenylimino)-1,3-dihydro-indol-3-one (**30**) and 3-(4-bromo phenylimino)-5-nitro-1,3-dihydro-indol-3-one (**13**) were found to be the most active compounds of the series. Mannich bases exhibited higher activity than the corresponding Schiff bases. © 2001 Éditions scientifiques et médicales Elsevier SAS

isatin / hydrazone / Schiff base / Mannich base / antibacterial activity

1. Introduction

Isatin is an endogenous compound isolated in 1988 [1] and reported to possess a wide range of central nervous system activities [2, 3]. Isatin is the biologically active chemical produced by an *Alteromones* sp. strain inhabiting the surface of embryos of the cardiean shrimp *Palaemon macrodectylus*, which protects them from the pathogenic fungus *Lagenidium callinectes* [4]. Schiff bases and Mannich bases of isatin were reported to possess antibacterial [5–7], antifungal [8–10], antiviral [11–13], anti-HIV [14–16], antiprotozoal [17, 18], and antihelminthic [19, 20] activities. In continuation of our work on biologically active isatin derivatives [21–23], we report the synthesis of some hydrazones, Schiff and *N*-Mannich bases of isatins and their antibacterial properties.

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2. Chemistry

In the present study, various aromatic primary amines/hydrazines were subjected to reaction with isatin/5-substituted isatin [24] to form Schiff bases and hydrazones, respectively. A new series of the corresponding *N*-Mannich bases was synthesised by reacting them with formaldehyde and diphenyl amine (*figure 1*).

3. Biological investigation and discussion

All the synthesised compounds were screened for antibacterial activity against seven Gram (+) and seven Gram (−) bacterial strains. According to preliminary antibacterial screening by the paper disc method (*tables IV and V*), compounds **8**, **15**–**18**, **23**, **31** and **33** were found to be inactive against all the bacterial strains. The minimum inhibitory concentrations (MIC) of the active compounds were also determined. The antibacterial activity of the synthesised compounds against standard and pathological strains

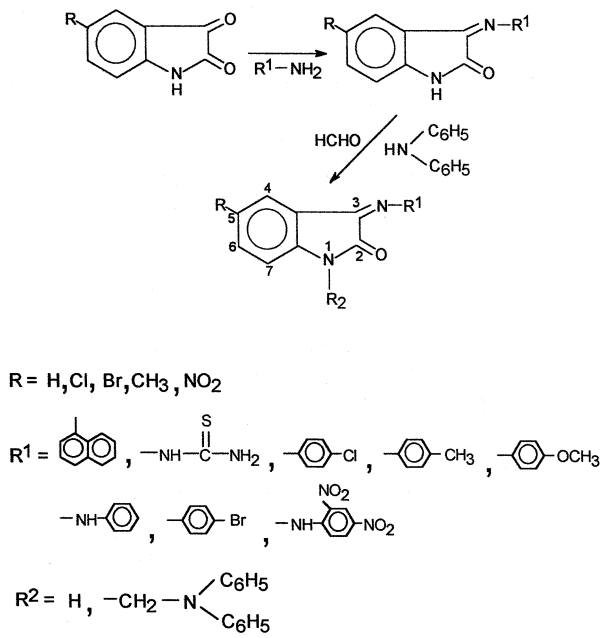

Figure 1. Protocol for the synthetic compounds.

was found to be similar (statistically insignificant difference). Most of the compounds exhibited mild to moderate antibacterial activity (*tables VI and VII*). All the compounds were effective against *Bacillus subtilis* ATCC 6051 (MIC: 18–304 μg mL^{-1}), *Pseudomonas aeruginosa* ATCC 2853 (4–172 μg mL^{-1}) and *Shigella dysenteriae* (64–214 μg mL^{-1}). Compounds **5**, **13**, **30**, **35**, **11**, and **24** exhibited highest activity against *Staphylococcus aureus* ATCC 25923 (94 μg mL^{-1}), *Staphylococcus epidermidis* ATCC 12228 (54 μg mL^{-1}), *Streptococcus pneumoniae* ATCC 49619 (31 μg mL^{-1}), *Bacillus cereus* ATCC 11778 (17 μg mL^{-1}), *B. subtilis* ATCC 6051 (18 μg mL^{-1}), *Bacillus pumilus* ATCC 14884 (24 μg mL^{-1}) and *Enterococcus faecalis* ATCC 29212 (104 μg mL^{-1}), respectively. Compounds **2**, **5**, **22**, **26**, **13** and **9** exhibited highest activity against *Escherichia coli* ATCC 25922 (12 μg mL^{-1}), *P. aeruginosa* ATCC 2853 (4 μg mL^{-1}), *Proteus vulgaris* ATCC 9484 (74 μg mL^{-1}), *Salmonella typhi* (7 μg mL^{-1}), *Salmonella typhimurium* ATCC 33068 (63 μg mL^{-1}), *S. dysenteriae* (64 μg mL^{-1}) and *Klebsiella pneumoniae* ATCC 13883 (194 μg mL^{-1}), respectively.

Compound **5** (4 μg mL^{-1}) was found to be more potent than Norfloxacin (20 μg mL^{-1}) against *P. aeruginosa*. All the active compounds were found to be more active than Amoxycillin against *P. aeruginosa*, *P. vulgaris* and *S. typhimurium*. Compounds **2** (12 μg mL^{-1}), **5** (114 μg mL^{-1}), **7** (67 μg mL^{-1}), **12** (41 μg mL^{-1}), **13** (66 μg mL^{-1}), **20** (40 μg mL^{-1}), **22** (18 μg mL^{-1}), **25** (14 μg mL^{-1}) and **26** (100 μg mL^{-1}) were found to be more active than Amoxycillin (128 μg mL^{-1}) against *E. coli*. 1-Diphenyl amino-methyl-3-(4-bromo phenylimino)-1,3-dihydro-indol-3-one (**30**) and 3-(4-bromo phenylimino)-5-nitro-1,3-dihydro-indol-3-one (**13**) were found to be the most active compounds against the screened Gram (+) and Gram (−) standard and pathological bacterial strains.

4. Experimental protocols

4.1. Chemistry

Melting points were determined in open capillary tubes in a Thomas–Hoover melting point apparatus and are uncorrected. IR spectra were recorded (in KBr) in a Bomem FTIR spectrophotometer M.B. Serial II. ^{1}H-NMR spectra were recorded in a 300 MHz Bruker DPX 200 spectrometer. The ^{1}H chemical shifts are reported as parts per million downfield from tetramethylsilane (Me_4Si). The purity of the compounds was checked by TLC on SiO_2 gel (HF_{254}, 200 mesh) coated glass plates using C_6H_6–$CHCl_3$ (55:45) visualised by iodine vapours. Microanalyses for C, H, N were performed in a Heraeus CHN Rapid Analyser, Division of Catalysis and Kinetics, Department of Chemistry, Indian Institute of Technology, Chennai, India. All the compounds gave satisfactory chemical analyses (±0.4%). Turbidity measurements were made in a Shimadzu 1601 UV–vis Spectrophotometer. ^{1}H-NMR and IR spectra were consistent with the assigned structures. The physico-chemical data are presented in *tables I–III*.

4.1.1. General procedure for Schiff bases and hydrazones of isatins

Equimolar quantities (0.004 mol) of isatin/5-substituted isatin and the aromatic primary amine/hydrazine were dissolved in 10 mL of warm ethanol and heated on a steam bath for 20–40 min. After standing for approximately 24 h at room temperature (r.t.), the crystalline products were separated by filtration, vacuum dried and recrystallised from ethanol.

Table I. Physico-chemical properties of the compounds.

Compound	R	R^1	Molecular formula	M.p. (°C)	Yield (%)	R_f value
1	H	1-naphthyl	$C_{18}H_{12}N_2O$	235	85	0.542
2	H	4-chloro phenyl	$C_{14}H_9N_2OCl$	240	62	0.880
3	H	4-bromo phenyl	$C_{14}H_9N_2OBr$	239	87	0.922
4	H	4-methoxy phenyl	$C_{15}H_{12}N_2O_2$	226	66	0.660
5	H	4-methyl phenyl	$C_{15}H_{12}N_2O$	222	66	0.662
6	H	phenyl hydrazino	$C_{14}H_{11}N_3O$	201	82	0.823
7	H	thiosemicarbazino	$C_9H_8N_4O$	198	53	0.900
8	H	2,4-dinitrophenylhydrazino	$C_{14}H_9N_5O_5$	222	77	0.774
9	CH_3	1-naphthyl	$C_{19}H_{14}N_2O$	217	72	0.540
10	CH_3	4-bromo phenyl	$C_{15}H_{11}N_2OBr$	228	77	0.955
11	CH_3	4-chloro phenyl	$C_{15}H_{11}N_2OCl$	211	44	0.877

Table II. Physico-chemical properties of the compounds.

Compound	R	R^1	Molecular formula	M.p. (°C)	Yield (%)	R_f value
12	NO_2	1-naphthyl	$C_{18}H_{11}N_3O_3$	218	60	0.522
13	NO_2	4-bromo phenyl	$C_{14}H_8N_3O_3Br$	223	72	0.900
14	NO_2	4-chloro phenyl	$C_{14}H_8N_3O_3Br$	220	70	0.866
15	NO_2	phenylhydrazino	$C_{14}H_{11}N_4O_3$	256	58	0.880
16	NO_2	2,4-dinitrophenylhydrazino	$C_{14}H_9N_6O_7$	179	70	0.744
17	CH_3	2,4-dinitrophenylhydrazino	$C_{15}H_{11}N_5O_5$	202	72	0.702
18	CH_3	4-methyl phenyl	$C_{16}H_{14}N_2O$	221	64	0.556
19	CH_3	4-methoxy phenyl	$C_{16}H_{14}N_2O_2$	246	72	0.421
20	CH_3	thiosemicarbazino	$C_{10}H_{10}N_4OS$	197	74	0.674
21	Cl	1-naphthyl	$C_{18}H_{11}N_2OCl$	219	78	0.799
22	Cl	4-chloro phenyl	$C_{14}H_8N_2OCl_2$	244	88	0.823
23	Cl	4-bromo phenyl	$C_{14}H_8N_2OClBr$	234	88	0.746

Table III. Physico-chemical properties of the compounds.

Compound	R	R^1	R^2	Molecular formula	M.p. (°C)	Yield (%)	R_f value
24	Cl	4-methoxy phenyl	H	$C_{15}H_{11}N_2O_2Cl$	254	90	0.714
25	Cl	4-methyl phenyl	H	$C_{15}H_{11}N_2OCl$	202	66	0.649
26	Cl	thiosemicarbazino	H	$C_9H_7N_4OSCl$	212	89	0.822
27	Br	1-naphthyl	H	$C_{18}H_{11}N_2OBr$	222	27	0.922
28	Br	4-methoxy phenyl	H	$C_{15}H_{11}N_2O_2Br$	264	23	0.917
29	H	1-naphthyl	CH_2–$N(C_6H_5)_2$	$C_{31}H_{23}N_3O$	236	88	0.835
30	H	4-bromo phenyl	CH_2–$N(C_6H_5)_2$	$C_{27}H_{20}N_3OBr$	202	75	0.742
31	H	4-methoxy phenyl	CH_2–$N(C_6H_5)_2$	$C_{28}H_{23}N_3O_2$	218	84	0.645
32	H	4-methyl phenyl	CH_2–$N(C_6H_5)_2$	$C_{28}H_{23}N_3O$	222	66	0.867
33	H	phenyl hydrazino	CH_2–$N(C_6H_5)_2$	$C_{27}H_{22}N_4O$	254	80	0.449
34	Cl	4-bromo phenyl	CH_2–$N(C_6H_5)_2$	$C_{27}H_{19}N_3OClBr$	247	42	0.880
35	Br	4-methoxy phenyl	CH_2–$N(C_6H_5)_2$	$C_{28}H_{22}N_3O_2Br$	200	48	0.499

*4.1.1.1. 3-(1-Naphthylimino)-1,3-dihydro-indol-2-one (**1**) [25]*

IR (KBr, cm^{-1}): 3150 (enolic O–H), 1630 (CO), 1590 (C=N), 1480, 1440 (C=C), 920, 910, 790, 760 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.34–6.72 (m, 3H, 5,6,7H), 6.77–7.13 (m, 2H, 2′, 8′H), 7.13–7.36 (d, 1H, 4H), 7.36–8.02 (m, 5H, 3′,4′,5′,6′,7′H), 8.30–8.72 (s, 1H, NH).

*4.1.1.2. 3-(4-Chloro-phenylimino)-1,3-dihydro-indol-2-one (**2**) [26]*

IR (KBr, cm^{-1}): 3220 (enolic O–H), 1625 (CO), 1590 (C=N), 1440 (C=C), 815, 780, 740 (Ar–H), 660 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.45–7.18 (m, 5H, 4,5,6,2′,6′H), 7.18–7.56 (m, 3H, 7,3′,5′,H), 8.92–9.39 (s, 1H, NH).

*4.1.1.3. 3-(4-Bromo-phenylimino)-1,3-dihydro-indol-2-one (**3**) [26]*

IR (KBr, cm^{-1}): 3220 (enolic O–H), 1625 (CO), 1590 (C=N), 1440 (C=C), 815, 780, 740 (Ar–H), 660 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.64–7.01 (m, 5H, 4,5,6,2′,6′H), 7.17–7.81 (m, 3H, 7,3′,5′,H), 8.95–9.30 (s, 1H, NH).

*4.1.1.4. 3-(4-Methoxy-phenylimino)-1,3-dihydro-indol-2-one (**4**) [26]*

IR (KBr, cm^{-1}): 3150 (enolic O–H), 1620 (CO), 1590 (C=N), 1440 (C=C), 1315 (C–H), 1230 (C–O), 815, 740 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.68–3.95 (s, 3H, 4′-OCH_3), 6.63–7.52 (m, 8H, 4,5,6,7,2′,3′,5′,6′H), 8.42–8.90 (s, 1H, NH).

*4.1.1.5. 3-(4-Methyl-phenylimino)-1,3-dihydro-indol-2-one (**5**) [26]*

IR (KBr, cm^{-1}): 3200 (enolic O–H), 1630 (CO), 1590 (C=N), 1440 (C=C), 1310 (C–H), 810, 735 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.26–2.53 (s, 3H, 4′-CH_3), 6.60–7.44 (m, 8H, 4,5,6,7,2′,3′,5′,6′H), 9.09–9.42 (s, 1H, NH).

*4.1.1.6. Indole-2,3-dione-3-phenyl hydrazone (**6**) [27]*

IR (KBr, cm^{-1}): 3100 (enolic O–H), 1660 (CO), 1530 (C=N), 1470, 1440 (C=C), 1220 (N–H), 735, 680 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.80–7.17 (m, 3H, 5,6,7H), 7.17–7.51 (m, 5H, 2′,3′,4′,5′,6′H), 7.51–7.74 (d, 1H, 4H), 7.80–8.13 (s, 1H, NH), 12.54–12.86 (s, 1H, –N=NH).

*4.1.1.7. Indole-2,3-dione-3-thiosemicarbazone (**7**) [28]*

IR (KBr, cm^{-1}): 3290 (N–H), 3100 (enolic O–H), 1650 (CO), 1600, 1570 (C=N), 1440 (C=C), 1185 (N–H), 1110 (C=S), 750, 730 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.84–7.04 (m, 3H, 5,6,7H), 7.07–7.23 (d, 1H, 4H), 7.48–7.75 (s, 2H, $–NH_2$), 7.77–8.05 (s, 1H, NH), 12.45–13.04 (s, 1H, –N=NH).

*4.1.1.8. 3-[(2,4-Dinitrophenylimino)-hydrazono]-1,3-dihydro-indol-2-one (**8**) [29]*

IR (KBr, cm^{-1}): 3200 (enolic O–H), 1590 (CO), 1560 (C=N), 1480, 1440 (C=C), 1320 (N=O), 1250 (N–H), 730, 680 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.50–3.62 (s, 3H, 5-CH_3), 6.12–6.35 (d, 1H, 2′H), 6.66–6.92 (d, 2H, 4′,5′H), 6.93–7.12 (d, 1H, 8′H), 7.12–7.60 (m, 6H, 4,6,7,3′,6′,7′H), 7.60–7.98 (s, 1H, NH).

*4.1.1.9. 3-(1-Naphthylimino)-5-methyl-1,3-dihydro-indol-2-one (**9**)*

IR (KBr, cm^{-1}): 3200 (enolic O–H), 1660 (CO), 1590 (C=N), 1460 (C=C), 1300 (C–H), 780, 760 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.10–2.63 (s, 3H, 5-CH_3), 6.47–6.66 (d, 2H, 2′,6′H), 6.68–6.85 (d, 1H, 7H), 6.87–7.00 (d, 1H, 6H), 7.04–7.34 (d, 2H, 3′,5′H), 7.38–7.48 (s, 1H, 4H), 7.48–7.62 (s, 1H, NH).

*4.1.1.10. 3-(4-Bromo-phenylimino)-5-methyl-1,3-dihydro-indol-2-one (**10**)*

IR (KBr, cm^{-1}): 3100(enolic O–H), 1700 (CO), 1590(C=N), 1470 (C=C), 1290 (C–H), 800, 720 (Ar–H), 630 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.02–1.90 (s, 3H, 5-CH_3), 6.52–6.71 (t, 2H, 4,6H), 6.73–6.87 (d, 1H, 7H), 6.92–7.05 (d, 2H, 2′,6′H), 7.06–7.19 (d, 2H, 3′,5′H), 8.12–8.67 (s, 1H, NH).

*4.1.1.11. 3-(4-Chloro-phenylimino)-5-methyl-1,3-dihydro-indol-2-one (**11**) [30]*

IR (KBr, cm^{-1}): 3000 (enolic O–H), 1690 (CO), 1590 (C=N), 1440 (C=C), 1290 (C–H), 800, 720 (Ar–H), 620 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.02–1.90 (s, 3H, 5-CH_3), 6.52–6.71 (t, 2H, 4,6H), 6.73–6.87 (d, 1H, 7H), 6.920–7.05 (d, 2H, 2′,6′H), 7.06–7.19 (d, 2H, 3′,5′H), 8.12–8.67 (s, 1H, NH).

*4.1.1.12. 3-(1-Naphthylimino)-5-nitro-1,3-dihydro-indol-2-one (**12**)*

IR (KBr, cm^{-1}): 3300 (enolic O–H), 1685 (CO), 1600 (C=N), 1490 (C=C), 1310 (N=O), 780, 755 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.67–6.87 (d, 1H, 8′H), 7.39–7.52 (m, 3H, 4,6,7H), 7.52–8.09 (m, 6H, 2′,3′,4′,5′,6′,7′H), 8.09–8.38 (s, 1H, NH).

*4.1.1.13. 3-(4-Bromo-phenylimino)-5-nitro-1,3-dihydro-indol-2-one (**13**) [30]*

IR (KBr, cm^{-1}): 3190 (enolic O–H), 1685 (CO), 1600 (C=N), 1470 (C=C), 1315 (N=O), 805, 740 (Ar–H), 630 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.42–6.67 (s, 1H, NH), 6.80–7.32 (m, 3H, 4,6,7H), 7.34–7.90 (d, 2H, 3′,5′H), 7.93–9.03 (d, 2H, 2′,6′H).

4.1.1.14. 3-(4-Chloro-phenylimino)-5-nitro-1,3-dihydro-indol-2-one ***(14)*** *[30]*

IR (KBr, cm^{-1}): 3200 (enolic O–H), 1690 (CO), 1600 (C=N), 1470 (C=C), 1310 (N=O), 805, 740 (Ar–H), 630 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.45–6.71 (m, 3H, 4,6,7H), 6.92–7.16 (m, 4H, 2′,3′,5′,6′H), 8.09–8.47 (s, 1H, NH).

4.1.1.15. 5-Nitro-indole-2,3-dione-3-phenyl hydrazone ***(15)***

IR (KBr, cm^{-1}): 3074 (enolic O–H), 1682 (CO), 1523 (C=N), 1447, 1420 (C=C), 1339 (N=O), 1225 (N–H), 745, 698, 680 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.95–7.73 (m, 8H, Ar–H), 7.80–8.13 (s, 1H, NH–CO), 12.54–12.87 (s, 1H, NH–Ar).

4.1.1.16. 3-[(2,4-Dinitrophenylimino)-hydrazono]-5-nitro-1,3-dihydro-indol-2-one ***(16)***

IR (KBr, cm^{-1}): 2915 (enolic O–H), 1616 (CO), 1522 (C=N), 1428 (C=C), 1316 (N=O), 1184 (N–H), 741, 677 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.94–8.14 (m, 7H, Ar–H), 8.04–8.34 (s, 1H, NH–CO), 12.56–12.88 (s, 1H, NH–Ar).

4.1.1.17. 3-[(2,4-Dinitrophenylimino)-hydrazono]-5-methyl-1,3-dihydro-indol-2-one ***(17)***

IR (KBr, cm^{-1}): 3166 (enolic O–H), 1618 (CO), 1592 (C=N), 1458, 1428 (C=C), 1313 (N=O), 1281 (C–H), 1223 (N–H), 740, 649 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.34–1.47 (s, 3H, 5-CH_3), 7.01–8.39 (s, 6H, Ar–H), 8.39–8.55 (s, 1H, NH–CO), 12.37–12.72 (s, 1H, NH–Ar).

4.1.1.18. 3-(4-Methyl-phenylimino)-5-methyl-1,3-dihydro-indol-2-one ***(18)*** *[31]*

IR (KBr, cm^{-1}): 3254 (enolic O–H), 1654 (CO), 1619 (C=N), 1478 (C=C), 1437, 1314 (C–H), 818, 731 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.97–2.57 (s, 6H, 5,4′-CH_3), 6.47–7.41 (m, 7H, Ar–H), 8.30–8.68 (s, 1H, NH–CO).

4.1.1.19. 3-(4-Methoxy-phenylimino)-5-methyl-1,3-dihydro-indol-2-one ***(19)***

IR (KBr, cm^{-1}): 3199 (enolic O–H), 1616 (CO), 1510 (C=N), 1441 (C=C), 1316 (C–H), 1289 (C–H), 1243 (C–O), 816, 770, 731 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.97–2.57 (s, 3H, 5-CH_3), 3.46–4.12 (s, 3H, 4′-OCH_3), 6.41–7.19 (m, 7H, Ar–H), 8.34–8.62 (s, 1H, NH–CO).

4.1.1.20. 5-Methyl-indole-2,3-dione-3-thiosemicarbazone ***(20)*** *[32]*

IR (KBr, cm^{-1}): 3300, 3343 (N–H), 3171 (enolic O–H), 1626 (CO), 1597 (C=N), 1436 (C=C), 1318 (C–H), 1209 (N–H), 1125 (C=S), 760 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.35–2.06 (s, 3H, 5-CH_3), 6.95–7.62 (m, 3H, Ar–H), 7.85–8.18 (s, 2H, 3′-NH_2), 8.27–8.68 (s, 1H, NH–CO), 12.32–12.53 (s, 1H, NH–Ar).

4.1.1.21. 3-(1-Naphthylimino)-5-chloro-1,3-dihydro-indol-2-one ***(21)***

IR (KBr, cm^{-1}): 2923 (enolic O–H), 1618 (CO), 1576 (C=N), 1457 (C=C), 805, 794, 767, 756 (Ar–H), 625 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.32–7.65 (m, 10H, Ar–H), 7.65–8.28 (s, 1H, NH–CO).

4.1.1.22. 3-(4-Chloro-phenylimino)-5-chloro-1,3-dihydro-indol-2-one ***(22)*** *[26]*

IR (KBr, cm^{-1}): 3249 (enolic O–H), 1644 (CO), 1584 (C=N), 1460 (C=C), 824, 717, 702 (Ar–H), 617, 517 (C=Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.15–7.62 (m, 7H, Ar–H), 8.14–8.58 (s, 1H, NH–CO).

4.1.1.23. 3-(4-Bromo-phenylimino)-5-chloro-1,3-dihydro-indol-2-one ***(23)*** *[26]*

IR (KBr, cm^{-1}): 3262 (enolic O–H), 1611 (CO), 1580 (C=N), 1461 (C=C), 829, 790, 748 (Ar–H); 669 (C–Br), 614 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.67–7.07 (s, 1H, NH), 7.13–7.73 (m, 7H, Ar–H).

4.1.1.24. 3-(4-Methoxy-phenylimino)-5-chloro-1,3-dihydro-indol-2-one ***(24)*** *[26]*

IR (KBr, cm^{-1}): 2924 (enolic O–H), 1609 (CO), 1595 (C=N), 1460 (C=C), 1252 (C–O), 1219 (C–H), 824, 756 (Ar–H), 669 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.64–3.80 (s, 3H, 4′-OCH_3), 6.53–6.89 (m, 7H, Ar–H), 6.89–7.18 (s, 1H, NH–CO).

4.1.1.25. 3-(4-Methyl-phenylimino)-5-chloro-1,3-dihydro-indol-2-one ***(25)*** *[26]*

IR (KBr, cm^{-1}): 3252 (enolic O–H), 1651 (CO), 1615 (C=N), 1461 (C=C), 1378 (C–H), 820, 752 (Ar–H), 611 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.97–2.57 (s, 3H, 4′-CH_3), 7.08–7.51 (m, 7H, Ar–H), 7.92–8.43 (s, 1H, NH–CO).

4.1.1.26. 5-Chloro-indole-2,3-dione-3-thiosemicarbazone ***(26)*** *[32]*

IR (KBr, cm^{-1}): 3414, 3318 (N–H), 3165 (enolic O–H), 1684 (CO), 1575 (C=N), 1466 (C=C), 1142

(N–H), 1122 (C=S), 784, 766 (Ar–H), 618, 606 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.12–6.41 (s, 1H, NH–CO), 6.46–6.74 (s, 2H, 3′-NH_2), 7.07–7.55 (m, 3H, Ar–H), 8.30–8.60 (s, 1H, NH–Ar).

4.1.1.27. 3-(1-Naphthylimino)-5-bromo-1,3-dihydro-indol-2-one ***(27)***

IR (KBr, cm^{-1}): 3200 (enolic O–H), 1615 (CO), 1578 (C=N), 1457 (C=C), 864, 813, 771, 751 (Ar–H), 650 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 6.40–6.94 (s, 1H, NH), 7.05–8.11 (m, 10H, Ar–H).

4.1.1.28. 3-(4-Methoxy-phenylimino)-5-bromo-1,3-dihydro-indol-2-one ***(28)*** *[30]*

IR (KBr, cm^{-1}): 3222 (enolic O–H), 1609 (CO), 1594 (C=N), 1460 (C=C), 1291 (C–H), 1254 (C–O), 819, 751 (Ar–H), 603 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.50–4.10 (s, 3H, 4′-OCH_3), 6.42–7.34 (m, 7H, Ar–H), 7.34–7.73 (s, 1H, NH).

4.1.2. General procedure for Mannich bases of isatins

Equimolar quantities (0.004 mol)of diphenyl amine in 10 mL of ethanol was added to a slurry containing the appropriate isatin and aqueous formaldehyde solution dissolved in 10 mL of ethanol. The reaction mixture was stirred for 1 h at r.t. and refrigerated for 48 h. The products were separated by suction filtration, vacuum dried and recrystallised from ethanol.

4.1.2.1. 1-Diphenylamino-methyl-3-(1-naphthylimino)-1,3-dihydro-indol-3-one ***(29)***

IR (KBr, cm^{-1}): 1748 (C=O), 1613 (C=N), 1461 (C=C), 1383 (–CH_2–), 799, 767, 750, 731, 689 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.30–1.95 (s, 2H, –CH_2–), 6.79–7.78 and 7.84–8.12 (m, 21H, Ar–H).

4.1.2.2. 1-Diphenylamino-methyl-3-(4-bromo-phenylimino)-1,3-dihydro-indol-3-one ***(30)***

IR (KBr, cm^{-1}): 1739 (C=O), 1610 (C=N), 1460 (C=C), 1477, 1393 (–CH_2–), 830, 811, 749, 688, 670 (Ar–H), 582 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.37–1.79 (s, 2H, –CH_2–), 6.65–8.49 (m, 18H, Ar–H).

4.1.2.3. 1-Diphenylamino-methyl-3-(4-methoxy-phenylimino)-1,3-dihydro-indol-3-one ***(31)***

IR (KBr, cm^{-1}): 1748 (C=O), 1613 (C=N), 1461 (C=C), 1326 (–CH_2–), 1280 (O–CH_3), 1268 (C–O), 799, 769, 689 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.46–1.74 (s, 2H, –CH_2–), 3.75–3.99 (s, 3H, 4′-OCH_3), 6.66–7.49 (m, 18H, Ar–H).

4.1.2.4. 1-Diphenylamino-methyl-3-(4-methyl-phenylimino)-1,3-dihydro-indol-3-one ***(32)***

IR (KBr, cm^{-1}): 1715 (C=O), 1645 (C=N), 1458 (C=C), 1390 (C–H), 1325 (–CH_2–), 819, 775, 746, 691 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.37–1.80 (s, 3H, 4′-CH_3), 2.09–2.62 (s, 2H, –CH_2–), 6.55–7.56 (m, 18H, Ar–H).

4.1.2.5. 1-Diphenylamino-methyl-indole-2,3-dione-3-phenyl hydrazone ***(33)***

IR (KBr, cm^{-1}): 1785 (C=O), 1685 (C=N), 1480 (C=C), 1465 (–CH_2–), 1243 (N–H), 789, 779, 746, 689, 662 (Ar–H). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.43–1.81 (s, 2H, –CH_2–), 6.30–6.74 and 6.80–6.99 and 7.00–8.05 (m, 17H, Ar–H).

4.1.2.6. 1-Diphenylamino-methyl-3-(4-bromo-phenylimino)-5-chloro-1,3-dihydro-indol-3-one ***(34)***

IR (KBr, cm^{-1}): 1748 (C=O), 1615 (C=N), 1458 (C=C), 1390 (–CH_2–), 689, 623 (Ar–H), 613 (C–Br), 579 (C–Cl). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.07–1.36 (s, 2H, –CH_2–), 6.37–7.95 (m, 17H, Ar–H).

4.1.2.7. 1-Diphenylamino-methyl-3-(4-methoxy-phenylimino)-5-bromo-1,3-dihydro-indol-3-one ***(35)***

IR (KBr, cm^{-1}): 1736 (C=O), 1609 (C=N), 1503 (C=C), 1459 (–CH_2–), 1291 (O–CH_3), 1253 (C–O), 838, 750, 700 (Ar–H), 602 (C–Br). ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.40–1.75 (s, 2H, –CH_2–), 3.65–4.04 (s, 3H, 4′-OCH_3), 6.59–7.58 (m, 7H, Ar–H).

4.2. Antibacterial screening

The standard strains were procured from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the Department of Microbiology, Madras Medical College and Research Institute, Chennai, India. The antibacterial activity of the synthesised compounds were screened against the following standard bacterial strains: *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. pneumoniae* ATCC 49619, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6051, *B. pumilus* ATCC 14884, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 2853, *P. vulgaris* ATCC 9484, *S. typhi*, *S. typhimurium* ATCC 33068, *S. dysenteriae* and *K. pneumoniae* ATCC 13883. The compounds were also screened against the following pathological bacterial strains: *S. aureus* (three strains), *B. cereus*, *B. subtilis*, *E. coli* (13 strains), *P. aeruginosa* (four strains), *P. vulgaris* (three strains), *S. typhi* (two strains) and *K. pneumoniae* (two strains).

Table IV. Antibacterial activity (paper disc diffusion method).

Compound	Gram positive bacteria—zone of inhibition (mm)						
	Staphylococcus aureus ATCC 25923	*Staphylococcus epidermidis* ATCC 12228	*Streptococcus pneumoniae* ATCC 49619	*Bacillus cereus* ATCC 11778	*Bacillus subtilis* ATCC 6051	*Bacillus pumilus* ATCC 14884	*Enterococcus faecalis* ATCC 29212
1	12	15	20	12	32	14	19
2	20	20	22	16	32	26	–
3	08	24	20	22	26	20	21
4	17	20	21	18	38	19	21
5	29	24	24	15	16	17	19
6	08	12	14	16	36	19	20
7	27	25	24	20	10	17	18
9	17	20	21	20	22	25	26
10	18	20	21	12	24	24	25
11	18	21	20	18	40	40	21
12	16	31	32	20	18	14	–
13	22	31	29	20	19	15	17
14	19	24	31	12	18	14	16
19	10	22	23	20	20	29	31
20	–	21	22	16	26	29	31
21	19	28	26	14	10	24	21
22	21	21	23	18	08	21	24
24	18	17	20	20	16	33	32
25	26	–	24	12	20	–	31
26	28	21	24	15	28	17	19
27	21	19	27	–	34	26	23
28	14	20	–	18	32	24	25
29	14	24	26	14	24	28	21
30	09	21	31	19	34	26	21
32	16	26	27	19	11	–	24
34	21	–	–	16	32	24	26
35	24	–	24	24	32	24	26

Table V. Antibacterial activity (paper disc diffusion method).

Compound	Gram negative bacteria—zone of inhibition (mm)						
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 2853	*Proteus vulgaris* ATCC 9484	*Salmonella typhi*	*Salmonella typhimurium* ATCC 33068	*Shigella dysenteriae*	*Klebsiella pneumoniae* ATCC 13883
1	18	24	19	19	20	21	34
2	28	36	23	25	24	31	30
3	18	17	–	18	14	15	–
4	–	32	16	19	–	20	24
5	21	46	17	26	18	21	30
6	20	28	20	20	18	21	32
7	24	38	24	25	18	21	32
9	28	34	27	–	20	23	22
10	–	08	15	10	11	14	12
11	19	11	19	20	16	17	09
12	25	28	–	26	17	18	–
13	24	35	24	24	18	23	35
14	18	36	22	19	25	31	30
19	10	21	14	11	18	20	17
20	25	21	16	16	–	24	24
21	–	26	16	09	12	17	10
22	22	35	21	28	26	25	09
24	–	44	–	–	23	24	29
25	28	39	22	20	25	24	28
26	22	36	22	25	40	34	12
27	18	29	14	12	21	20	12
28	18	30	15	10	20	17	12
29	–	26	24	14	12	21	24
30	–	18	21	12	08	09	10
32	20	24	14	10	21	15	21
34	18	21	21	16	13	16	21
35	18	26	–	–	17	18	30

Table VI. Antibacterial activity (minimum inhibitory concentration).

Compound	Gram positive bacteria—minimum inhibitory concentration ($\mu g\ mL^{-1}$)						
	Staphylococcus aureus ATCC 25923	*Staphylococcus epidermidis* ATCC 12228	*Streptococcus pneumoniae* ATCC 49619	*Bacillus cereus* ATCC 11778	*Bacillus subtilis* ATCC 6051	*Bacillus pumilus* ATCC 14884	*Enterococcus faecalis* ATCC 29212
1	169	189	200	142	54	164	262
2	139	162	164	66	53	62	–
3	206	109	198	27	108	107	218
4	136	154	178	53	29	106	219
5	94	110	102	73	259	139	260
6	204	204	267	67	36	107	229
7	106	88	104	34	290	141	274
9	134	160	201	35	169	68	149
10	130	159	202	141	158	74	154
11	131	156	176	52	18	24	217
12	145	56	32	35	108	166	–
13	120	54	56	35	217	156	299
14	106	106	32	138	240	162	324
19	196	124	164	36	198	47	114
20	–	156	172	66	198	46	115
21	131	67	89	104	284	72	220
22	127	151	134	54	304	81	172
24	206	180	202	36	258	34	104
25	105	–	88	136	196	–	113
26	102	150	102	73	69	139	260
27	126	177	69	–	48	61	204
28	151	160	–	54	52	72	151
29	150	108	88	102	156	53	214
30	198	151	31	42	47	60	216
32	146	74	68	41	273	–	170
34	120	–	–	66	53	72	147
35	116	–	104	17	55	73	148
Amoxycillin	16	6	<2	2	2	2	2
Norfloxacin	16	3	16	<2	<2	<2	10

Table VII. Antibacterial activity (minimum inhibitory concentration).

Compound	Gram negative bacteria—minimum inhibitory concentration ($\mu g\,mL^{-1}$)						
	Escherichia coli ATCC 25922	*Pseudomonas aeruginosa* ATCC 2853	*Proteus vulgaris* ATCC 9484	*Salmonella typhi*	*Salmonella typhimurium* ATCC 33068	*Shigella dysenteriae*	*Klebsiella pneumoniae* ATCC 13883
1	166	120	131	49	134	69	324
2	12	39	110	21	79	84	242
3	165	151	–	94	190	179	–
4	–	71	130	152	–	124	378
5	114	04	90	62	133	83	346
6	131	84	129	54	167	111	300
7	67	34	111	63	133	74	218
9	140	67	–	–	121	139	194
10	–	172	188	149	195	186	390
11	138	166	128	88	171	214	323
12	41	82	91	75	167	85	–
13	66	47	120	62	120	64	218
14	165	37	130	17	78	83	277
19	266	134	181	62	150	167	402
20	40	135	141	139	–	123	378
21	–	94	194	137	170	201	317
22	98	46	74	10	98	213	280
24	–	22	–	–	108	96	288
25	14	29	130	16	107	109	276
26	100	40	110	07	63	185	275
27	165	80	170	34	149	186	400
28	164	74	187	48	169	187	388
29	–	93	162	137	131	122	216
30	–	149	171	164	202	200	288
32	130	119	188	32	188	151	402
34	164	132	140	112	184	150	281
35	165	92	–	74	166	83	209
Amoxycillin	128	1020	512	2	512	26	128
Norfloxacin	<2	20	<2	<2	<2	<2	20

4.2.1. Paper disc diffusion method

Preliminary antibacterial screening [33] was performed by the agar diffusion method using a paper disc. The sterilised (autoclaved at 120 °C for 30 min), liquefied mueller hinton agar (40–50 °C) was inoculated (1 mL/100 mL of medium) with the suspension of the microorganism (matched to McFarland Barium sulphate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper discs impregnated with the test compounds (500 mg mL^{-1} in dimethyl sulphoxide) were placed on the solidified medium. The plates were refrigerated for 2 h at 4 °C and then incubated at 37 °C for 24 h. The observed zones of inhibition are presented in *tables IV and V*.

4.2.2. Minimum inhibitory concentration

A series of glass tubes [34] containing different concentrations of the synthesised compounds (in dimethyl sulphoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism which contains 10^5 colony forming units per millilitre. One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was recorded by using a UV–visible spectrometer. The minimum inhibitory concentration (MIC—μg mL^{-1}) was considered to be the lowest concentration which exhibited the same turbidity as the blank tube. The observed MICs (μg mL^{-1}) are presented in *tables VI and VII*.

References

[1] Glover V., Halket J.M., Watkins P.J., Clone A., Goodwin B.L., Sandler M., J. Neurochem. 51 (1988) 656–660.

[2] Bhattacharya S.K., Glover Vivette, McIntyre I., Oxenkrug G., Sandler M., Neurosci. Lett. 92 (1982) 218–221.

[3] Bhattacharya Salil K., Mitra Shankar K., Acharya Satya B., J. Psychopharmacol. 5 (1991) 202–206.

[4] Gil-Turners M.S., Hay M.E., Fenical W., Science 246 (1989) 116–118.

[5] Pandeya S.N., Sriram D., Acta Pharm. Turc. 40 (1998) 33–38.

[6] Sarangapani M., Reddy V.M., Indian J. Pharm. Sci. 56 (1994) 174–177.

[7] Varma R.S., Nobles W.L., J. Pharm. Sci. 64 (1975) 881–882.

[8] Pandeya S.N., Sriram D., Nath G., De Clercq E., Indian J. Pharm. Sci. 61 (1999) 358–361.

[9] Pandeya S.N., Sriram D., Nath G., De Clercq E., Sci. Pharm. 67 (1999) 103–111.

[10] Pandeya S.N., Sriram D., Nath G., De Clercq E., Pharm. Acta Helv. 74 (1999) 11–17.

[11] Varma R.S., Nobles W.L., J. Med. Chem. 10 (1967) 972–974.

[12] Singh S.P., Shukla S.K., Awasthi L.P., Curr. Sci. 52 (1983) 766–769.

[13] Logan J.C., Fox M.P., Morgan J.M., Makohon A.M., Pfau C.J., J. Gen. Virol. 28 (1975) 271–283.

[14] Pandeya S.N., Yogeeswari P., Sriram D., De Clercq E., Pannecouque C., Witvrouw M., Chemotherapy 45 (1999) 192–196.

[15] Pandeya S.N., Sriram D., Nath G., De Clercq E., Eur. J. Med. Chem. 35 (2000) 249–255.

[16] Pandeya S.N., Sriram D., Nath G., De Clercq E., Arzneimittel-Forschun./Drug Res. 50 (2000) 55–59.

[17] Imam S.A., Varma R.S., Experientia 31 (1975) 1287–1288.

[18] Varma R.S., Khan I.A., Polish J. Pharmacol. Pharm. 29 (1977) 549–594.

[19] Sarciron S.E., Audin P., Delebre I., Gabrion C., Petavy A.F., Paris J., J. Pharm. Sci. 82 (1993) 605–609.

[20] Et-Sawi E.A., Mostafa T.B., Mostafa B.B., J. Egypt. Soc. Parasitol. 28 (1998) 481–486.

[21] Sridhar S.K., Pandeya S.N., Bajpai S.K., Manjula H., Indian Drugs 36 (1999) 412–414.

[22] Sridhar S.K., Ramesh A., Indian J. Chem., Sec. B, in press.

[23] Sridhar S.K., Ramesh A., Indian Drugs, in press.

[24] Marvel C.S., Hiera G.S., Organic Synthesis, Wiley, New York, 1951, p. 327.

[25] Knoevenagel J., Prakt. Chem. (1914) 48.

[26] Varma R.S., Garg P.K., J. Indian Chem. Soc. 58 (1981) 980–981.

[27] Katritzky A.R., Fan W.Q., Koziol A.E., Palenik G.J., J. Heterocycl. Chem. 26 (1989) 821–828.

[28] Somogyi L., Liebigs Ann. (1993) 931–934.

[29] Abdel-Rahman R.M., Islam I.E., El-Gendy Z., J. Indian Chem. Soc. 68 (1991) 621–624.

[30] Rajopadhye M., Popp F.D., J. Heterocycl. Chem. 21 (1984) 289–291.

[31] Meyer P., Chem. Ber. 16 (1883) 2262.

[32] Gupta N., Singh R.V., Indian J. Chem., Sec. A 37 (1998) 75–79.

[33] Gillespie S.H., Medical Microbiology—Illustrated, Butterworth Heinemann, London, 1994, pp. 234–247.

[34] Hawkey P.M., Lewis D.A., Medical Bacteriology—A Practical Approach, Oxford University Press, Oxford, 1994, pp. 181–194.