Synthesis and antimicrobial activity of 1-aminomethyl-3-[4\(^\prime\)-\(4\text{"}-nitrobenzyl-\)oxy]-benzohydrazono]isatins

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A new series of 1-aminomethyl-3-[4\(^\prime\)-\(4\text{"}-nitrobenzyl-\)oxy]-benzohydrazono]isatins (Mannich bases) have been synthesized and screened for their antimicrobial potential against human pathogenic bacteria and fungi. The structures of the compounds have been established by means of elemental analysis and spectral data (IR and \(^1\)H NMR).

Keywords: Isatin, Schiff base, Mannich base, antimicrobial activity

Isatins\(^1\) and their derivatives have been reported to possess wide variety of biological activities viz; cytotoxic\(^2\)-6, antimicrobial\(^7\)-11, antiviral\(^12\), anti-fertility\(^13\), amoebicidal\(^14\), anti-HIV\(^15\), antileukemic\(^16\), anticonvulsant\(^17\), CNS-depressant\(^18\)-20, analgesic and anti-inflammatory\(^21\). Recently two reviews\(^22\),\(^23\) have been published on the biological potential of isatins. Keeping this biological activity profile in mind, a new series of Mannich bases is being reported here.

4-(4\(^\prime\)-Nitrobenzyl-\)oxy)-benzohydrazide 2 was prepared by hydrazinolysis of methyl 4-(4\(^\prime\)-nitrobenzyl-\)oxy)-benzoate 1 which in turn was obtained by O-benzylation of methyl 4-hydroxybenzoate with 4-nitrobenzyl bromide. Benzohydrazide 2 on condensation with isatins in equimolar proportion, gave 3-[4\(^\prime\)-(4\(^\prime\)-nitrobenzyl-\)oxy]-benzohydrazono]isatins (Schiff base) 3-5. Compound 3 on being subjected to aminomethylation\(^24\) with heterocyclic secondary amines in the presence of formaldehyde, gave 1-aminomethyl-3-[4\(^\prime\)-(4\(^\prime\)-nitrobenzyl-\)oxy]-benzohydrazono]isatins (Mannich bases) 6-14 (Scheme I).

### Antimicrobial activity

Compounds 3-14 were screened for their in-vitro antimicrobial potential against human pathogenic bacteria viz; *Escherichia coli* (ATCC 9637) (EC), *Pseudomonas aeruginosa* (ATCCBAA 427 PA), *Staphylococcus aureus* (ATCC 25923) (SA), *Klebsiella pneumoniae* (ATCC 27736) (KP) and fungi *Candida albicans* (CA), *Cryptococcus neoformans* (CN), *Trichophyton mentagrophytes* (TM), *Aspergillus fumigatus* (AF) using tube dilution method at maximum concentration of 50 µg/mL and minimum inhibitory concentration (MIC) values were determined in µg/mL. Gentamycin and fluconazole were taken as standard drugs for bacteria and fungi respectively. Antimicrobial activity data are presented in Table I.

### Experimental Section

The melting points were obtained in open capillaries in sulfuric acid bath and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer RX1 spectrophotometer and \(^1\)H NMR on Bruker Avance 400 spectrometer. CDCl\(_3\)/DMSO-\(d_6\) were used as solvent and TMS as internal reference. Chemical shifts are expressed in \(\delta\) (ppm). Elemental analysis data were obtained on Carlo Erba 1108 analyser. Homogeneity of the compounds were checked by TLC silica gel G plates and spots were located by exposure to iodine vapors.

**Methyl 4-(4\(^\prime\)-nitrobenzyl-\)oxy)-benzoate, 1**

A mixture of methyl 4-hydroxybenzoate (0.045 mol), 4-nitrobenzyl bromide (0.045 mol) and anhyd. K\(_2\)CO\(_3\) (7.1 g) in acetone (70 mL) was refluxed for 9-10 hr. Excess of solvent was distilled off and the contents were poured into cold water. The solid so obtained was filtered, washed with water, dried and purified by recrystallization from 1-propanol. m.p.182-84°C, Yield 78%; IR (KBr): 1708 (CO), 1529, 1350 (NO\(_2\)), 1261 cm\(^{-1}\)(-CH\(_2\)O-).

**4-(4\(^\prime\)-Nitrobenzyl-\)oxy)-benzohydrazide, 2**

Compound 1 (0.01 mol) and hydrazine hydrate (99%, 0.01 mol) in 1-propanol (100 mL) were refluxed for 24 hr. Excess of solvent was distilled off.
Table I — Minimum Inhibitory Concentration, MIC (µg/mL) of compounds against bacteria and fungi

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EC = Escherichia coli, PA = Pseudomonas aeruginosa, SA = Staphylococcus aureus, KP = Klebsiella pneumoniae, CA = Candida albicans, CN = Cryptococcus neoformans, TM = Trichophyton mentagrophytes, AF = Aspergillus fumigatus.

(i) 4-Nitrobenzyl bromide R = H, Me, COCH₃
(ii) K₂CO₃(anhyd); Me₂CO R’ = morpholino, piperidino, pyrrolidino
(iii) N₂H₄·H₂O; 1-Propanol N-methylpiperazino, N-ethylpiperazino
(iv) Isatins, gl. AcOH; EtOH N-phenylpiperazino, N-benzylpiperazino
(v) Amines, CH₃O; DMF dimethylamino, diethylamino

Scheme I
and poured into water. The solid thus obtained was filtered, washed with water, dried and purified by recrystallization from 1-propanol. m.p. 202-04°C, Yield 84%; IR (KBr): 3315, 3179 (NHNHz), 1659 (CONH), 1527, 1350 (NO2), 1255 cm⁻¹ (-CH₂-O-).

3-[4′-′(4′-Nitrobenzyl)oxy]-benzohydrazono]isatin, 3
A mixture of 4-(4-nitrobenzyl)-benzohydrazide 2 (0.01 mol) and isatin (0.01 mol) in ethanol (50 mL) containing 2-3 drops of glacial acetic acid was refluxed for 1 hr and left overnight at RT. The separated solid was filtered and washed with methanol. m.p.>280°C, Yield 74%; IR (KBr): 3480, 3170 (NH), 1691, 1662 (CO), 1528, 1345 (NO2), 1254 cm⁻¹ (-CH₂-O-). Anal. Found: C, 63.42; H, 3.80; N, 13.40. C₂₂H₁₈N₆O₅ requires: C, 63.46; H, 3.84; N, 13.46%.

Compounds 4 and 5 were synthesized by similar methods using N-methyl and N-acetyl isatins.

1-Methyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 4
m.p. 228-30°C, Yield 86%; ¹H NMR (DMSO-d₆): δ 2.63 (s, 3H, N-Me), 5.25 (s, 2H, -CH₂-O-), 6.96-8.02 (m,12H, Ar-H), 13.98 (s,1H, NHCO). Anal. Found: C, 64.12; H, 4.13; N, 12.95. C₂₂H₁₈N₆O₅ requires: C, 64.18; H, 4.18; N, 13.02%.

1-Acetyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 5
m.p. 240-42°C, Yield 73%; ¹H NMR (DMSO-d₆): δ 2.32 (s, 3H, COCH₃), 5.27 (s, 2H, -CH₂-O-), 6.94-8.07 (m,12H, Ar-H), 13.89 (s, 1H, NHCO). Anal. Found: C, 62.82; H, 3.88; N, 12.18. C₂₂H₁₈N₆O₅ requires: C, 62.88; H, 3.93; N, 12.22%.

1-Morpholinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 6
To a suspension of 3 (0.005 mol) in DMF, formaldehyde (0.5 mL, 37% aqu. solution) and morpholine (0.005 mol) was added with vigorous stirring, warmed for 2 min on a water bath and left overnight at RT. The solid product thus obtained was filtered, washed with methanol, dried and purified by recrystallization from chloroform: pet.-ether (60-80°C) (1:1). m.p.188-90°C, Yield 74%; IR (KBr): 3480 (NH), 2804 (>N-CH₂-N<), 1682 (CO), 1529, 1349 (NO2), 1255 cm⁻¹ (-CH₂-O-); ¹H NMR (CDCl₃): δ 2.63-2.67 (t, 4H, -CH₂-N⁻CH₂-), 3.70-3.73 (t, 4H, -CH₂-O⁻CH₂-), 4.47 (s, 2H, >N-CH₂-N<), 5.25 (s,2H, -CH₂-O-), 6.77-8.12 (m,12H, Ar-H), 13.77 (s,1H, NHCO). Anal. Found: C, 62.88; H, 4.80; N, 13.55. C₂₅H₂₁N₉O₅ requires: C, 62.91; H, 4.85; N, 13.59%.

Mannich bases 7-14 were synthesized using same procedure.

1-Piperidinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 7
m.p. 212-14°C, Yield 68%; IR (KBr): 3456 (NH), 2853 (>N-CH₂-N<), 1686 (CO), 1521, 1349 (NO2), 1250 cm⁻¹ (-CH₂-O-); ¹H NMR (CDCl₃): δ 1.45-1.52 (m, 6H, -CH₂CH₂CH₂-), 2.53-2.59 (t, 4H, -CH₂-N⁻CH₂-), 4.51 (s, 2H, >N-CH₂-N<), 5.25 (s, 2H, -CH₂-O-), 7.05-8.12 (m, 12H, Ar-H), 13.87 (s,1H, NHCO). Anal. Found: C, 65.44; H, 5.21; N, 13.59. C₂₉H₂₇N₉O₅ requires: C, 65.49; H, 5.26; N, 13.64%.

1-Pyrrolidinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 8
m.p. 202-04°C, Yield 70%; IR (KBr): 3505 (NH), 2820 (>N-CH₂-N<), 1678 (CO), 1522, 1345 (NO2), 1251 cm⁻¹ (-CH₂-O-); ¹H NMR (CDCl₃): δ 1.35-1.42 (m, 4H-CH₂CH₂-), 2.33-2.46 (t, 4H, -CH₂-N⁻CH₂-), 4.54 (s,2H, >N-CH₂-N<), 5.25 (s,2H, -CH₂-O-), 7.05-8.15 (m,12H, Ar-H), 13.83 (s,1H, NHCO). Anal. Found: C, 64.87; H, 4.97; N, 13.96. C₃₀H₂₉N₉O₅ requires: C, 64.92; H, 5.10; N, 14.02%.

1-N-Methylpiperazinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 9
m.p. 208-10°C, Yield 65%; ¹H NMR (CDCl₃): δ 1.87 (s, 3H, N-Me), 2.33-2.39 (t, 4H, -CH₂-N⁻CH₂-), 2.56-2.65 (t, 4H, -CH₂N(Me)CH₂-), 4.55 (s,2H, >N-CH₂-N<), 5.25 (s, 2H, -CH₂-O-), 6.88-7.99 (m,12H, Ar-H), 14.00 (s,1H, NHCO). Anal. Found: C, 63.58; H, 5.28; N, 15.87. C₂₉H₂₉N₉O₅ requires: C, 63.63; H, 5.30; N, 15.90%.

1-N-Ethylpiperazinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 10
m.p. 176-78°C, Yield 56%; ¹H NMR (CDCl₃): δ 1.04-1.07 (t, 3H, CH₃CH₂), 1.72-1.89 (t, 4H, -CH₂-N⁻CH₂-), 2.37-2.41 (q, 2H, -CH₂CH₃), 2.43-2.71 (t, 4H, -CH₂N(ETH)CH₂-), 4.54 (s, 2H, >N-CH₂-N<), 5.25 (s, 2H, -CH₂-O-), 7.04-8.28 (m,12H, Ar-H), 13.96 (s, 1H, NHCO). Anal. Found: C, 64.17; H, 5.48; N, 15.45. C₃₀H₃₀N₉O₅ requires: C, 64.20; H, 5.53; H, 15.49%.

1-N-Phenylpiperazinomethyl-3-[4′-′(4′-nitrobenzyl)oxy]-benzohydrazono]isatin, 11
m.p. 212-14°C, Yield 56%; IR (KBr): 3453 (NH), 2826 (>N-CH₂-N<), 1683 (CO), 1529, 1346 (NO₂),
1244 cm⁻¹ (-CH₂O⁻); ¹H NMR (CDCl₃): δ 2.33-2.41 (t, 4H, -CH₂-N-CH₂-), 2.46-2.65 (t, 4H, -CH₂-N(Ph)-CH₂-), 4.55 (s, 2H, >N-CH₂-N<), 5.25 (s, 2H, -CH₂O⁻), 7.04-8.28 (m,17H, Ar-H), 14.00 (s, 1H, NHCO). Anal. Found: C, 67.08; H, 4.98; N, 14.18. C₃₃H₃₀N₆O₅ requires: C, 67.11; H, 5.08; N, 14.23%.

1-N-Benzylpiperazinomethyl-3-[4′-(4′′-nitrobenzyloxy)-benzohydrazono]isatin, 12

m.p. 206-08°C, Yield 60%; ¹H NMR (CDCl₃): δ 2.33-2.48(t, 4H, -CH₂-N-CH₂-), 2.62-2.69 (t, 4H, -CH₂N(CH₂Ph)CH₂-), 3.49 (s, 2H,-CH₂Ph), 4.55 (s, 2H, -CH₂O⁻), 7.04-8.28 (m,17H, Ar-H), 14.01 (s, 1H, NHCO). Anal. Found: C, 64.63; H, 5.35; N, 13.94. C₂₇H₂₅N₆O₅ requires: C, 64.67; H, 5.38; N, 13.97%.

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References