Synthesis and Antifungal Activity of 1-Aminomethyl-3-[4''-(4'''-fluorobenzyloxy) -benzohydrazono] isatins

Nisheeth Rastogi*
Department of Chemistry, Dr. W. E. Bauer Research Laboratory, Lucknow Christian Degree College, Lucknow, Uttar Pradesh, India

ABSTRACT A new series of 1-aminomethyl-3-[4''-(4'''-fluorobenzyloxy)-benzohydrazono] isatins (Mannich bases) have been synthesized and screened for their antifungal potential against human pathogenic fungi. The structures of the compounds have been established by means of elemental analysis and spectral data (infrared and $^1$H nuclear magnetic resonance).

INTRODUCTION Isatins$^{[1]}$ and their derivatives have been reported to possess wide variety of biological activities, namely anticancer,$^{[2]}$ antitubercular,$^{[3]}$ antileishmanial,$^{[4]}$ antimicrobial,$^{[5]}$ anticonvulsant,$^{[6]}$ antiviral,$^{[7]}$ antioxidant,$^{[8]}$ anti-inflammatory,$^{[9]}$ and analgesic.$^{[10]}$ During the past two decades, large number of review$^{[11]}$ articles have been published on chemistry and biological potential of isatins. In the light of these articles, a new series of Mannich bases of isatins is being reported here.

RESULTS AND DISCUSSION Chemistry

4-(4''-Fluorobenzyloxy)-benzohydrazide 2 was prepared by the hydrazinolysis of methyl 4-(4''-fluorobenzyloxy)-benzoate 1 which, in turn, was obtained by O-benzylation of methyl-4-hydroxybenzoate with 4-fluorobenzyl chloride. Benzohydrazide 2 on condensation with isatin/N-substituted isatins in equimolar proportion gave 3-[4''-(4'''-fluorobenzyloxy)-benzohydrazono] isatin/1-substituted-3-[4''-(4'''-fluorobenzyloxy)-benzohydrazono] isatins (Schiff bases) 3-7. Compound 3 on being subjected to aminomethylation$^{[12]}$ with secondary amines (aliphatic and heterocyclic) in the presence of formaldehyde gave 1-aminomethyl-3-[4''-(4'''-fluorobenzyloxy)-benzohydrazono] isatins (Mannich bases) 8-19 [Scheme-1].

Antifungal activity

All the compounds 3-19 were screened for their in vitro antifungal potential against human pathogenic fungi, namely Candida albicans (CA), Cryptococcus neoformans.

*Corresponding author: Email:nisheethrastogi2003@gmail.com
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(CN), Candida parapsilosis (CP), Trichophyton mentagrophytes (TM), and Aspergillus fumigatus (AF), using tube dilution method at a maximum concentration of 100 μg/mL in dimethyl sulfoxide (DMSO). Antifungal activity data are presented in Table 1. Schiff bases were found to be inactive or showed minimum inhibitory concentration (MIC) of no significance against all the tested fungi. However, on aminomethylation which resulted into Mannich bases, compounds showed good to weak activity against fungi. Mannich bases (8, 9, and 11) with morpholinomethyl, piperidinomethyl, and pyrrolidinomethyl groups showed activity against CN, TM, and AF with MIC 3.12 μg/mL. Compounds 12, 13, and 16 showed MIC 3.12 against TM and AF, while 17, 18, and 19 showed MIC 3.12 against TM only. Mannich bases were found to be good to moderately active against TM and AF while they were found to inactive against CA and CP. The trend of antifungal activity of Mannich bases was so diverse that no structure-activity relationship could be established in terms of amines.

EXPERIMENTAL SECTION

The melting points were determined in open capillary tubes in sulfuric acid bath and are uncorrected. Infrared (IR) spectra were recorded in KBr on a Perkin Elmer spectrophotometer and frequencies are presented as cm⁻¹. ¹H nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 300 spectrometer using DMSO-d₆/CDCl₃ as solvent and TMS as internal reference. Chemical shift values are expressed in δ (ppm). Elemental analysis data were obtained on Carlo Erba 1108 analyzer. Homogeneity of the compounds was checked on TLC silica gel G plates and spots were located by exposure to iodine vapors.

Table 1: Minimum inhibitory concentration in μg/mL of compounds against fungi

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<th>Compound</th>
<th>Candida albicans</th>
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<th>Trichophyton mentagrophytes</th>
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Scheme 1

(a) 4-Fluorobenzyl chloride, anhyd. K₂CO₃, Me₂CO
(b) N₂H₄·H₂O, 1-Propanol
(c) Isatin/ N-substituted isatins, EtOH, gl. AcOH
(d) Amines, CH₃OH, DMF

R=H, Me, COMe, CH₃OH, COPh
R’=Morpholinomethyl, piperidinomethyl, 2-methylpiperidinomethyl, pyrrolidinomethyl, N-methylpiperazinomethyl, N-ethylpiperazinomethyl, N-phenylpiperazinomethyl, N-benzylpiperazinomethyl, dimethylaminomethyl, diethylaminomethyl, di-n-propylaminomethyl, diisopropylaminomethyl
Methyl 4-(4'-fluorobenzyloxy)-benzoate, 1

A mixture of methyl 4-hydroxybenzoate (0.045 mol), 4-fluorobenzyl chloride (0.045 mol), and anhyd. K₂CO₃ (7.1 g) in acetone (60 mL) was refluxed for 8–9 h. 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. 94–96°C; yield 86%; IR(KBr): 1720 (CO), 1256 (CH₂O), 1050 cm⁻¹ (C-F).

4-(4'-Fluorobenzyloxy)benzohydrazide, 2

Methyl 4-(4'-fluorobenzyloxy)-benzoate 1 (0.01 mol) and hydrazine hydrate (99%, 1 mL) in 1-propanol (100 mL) were refluxed for 15 h. Excess of solvent was distilled off and contents were poured into water. The solid thus obtained was filtered, washed with water, dried, and purified by recrystallization from 1-propanol. m.p. 154–56°C; yield 78%; IR(KBr): 3324, 3198 (NHNH₂), 1677 (CONH), 1255 (C-F). Anal. found: C, 65.81; H, 4.29; N, 9.96. C₂₇H₂₅FN₃O₄ requires: C, 65.87; H, 4.33; N, 10.02%.

3-[4'-(4'-Fluorobenzyloxy)benzohydrazono]isatin, 3

A mixture of 4-(4'-fluorobenzyloxy)benzohydrazide 2 (0.01 mol) and isatin (0.01 mol) in ethanol (70 mL) containing 2–3 drops of glacial acetic acid was refluxed for 2 h and left overnight at room temperature. The separated solid was filtered and washed with methanol. m.p. 239–241°C; yield 68%; IR(KBr): 3424, 3180 (NH), 1677 (CO), 1256 (C-F). Anal. found: C, 68.41; H, 4.45; N, 10.70. C₂₈H₂₇FN₄O₃ requires: C, 68.48; H, 4.50; N, 10.42%.

1-Methyl-3-[4'-(4'|-fluorobenzyloxy)-benzohydrazono]isatin, 4

m.p. 180°C; yield 78%; IR(KBr): 3477 (NH), 2809 (>N-CH₂-N<), 1682 (CO), 1253 (CH₂O), 1060 cm⁻¹ (C-F). Anal. found: C, 67.77; H, 4.10; N, 10.70. C₂₉H₂₆FN₄O₃ requires: C, 67.86; H, 4.14; N, 10.79%.

Compounds 4-7 were synthesized by similar method using N-methyl, N-acetyl, N-hydroxymethyl, and N-benzoyl isatins.

1-Benzoyl-3-[4'-(4'|-fluorobenzyloxy)-benzohydrazono]isatin, 7

m.p. 179–80°C; yield 68%; IR(KBr): 5.26 (s, 2H, >N-CH₂-O-), 6.90–8.15 (m, 17H, Ar-H), 13.89 (s, 1H, NHCO); Anal. found: C, 70.53; H, 4.00; N, 8.49. C₂₇H₂₆FN₄O₄ requires: C, 70.58; H, 4.08; N, 8.51%.

1-Morpholinomethyl-3-[4'-(4'-fluorobenzyloxy)-benzohydrazono]isatin, 8

To a suspension of 3 (0.005 mol) in DMF, formaldehyde (0.5mL, 37% eq. solution) and morpholine (0.005 mol) were added with vigorous stirring, and warmed for 2 min on a water bath, and left overnight at room temperature. 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. 164–66°C; yield 70%; IR (KBr): 3477 (NH), 2809 (>N-CH₂-N<), 1682 (CO), 1253 (CH₂O), 1060 cm⁻¹ (C-F); 'H NMR (CDCl₃): δ 1.92–2.65 (t, 4H, >N-CH₂-N<), 3.65–3.68 (t, 4H, >N-CH₂-O-), 4.44 (s, 2H, >N-CH₂-N<), 5.28 (s, 2H, >N-CH₂-O-), 6.78–8.05 (m, 12H, Ar-H), 13.80 (s, 1H, NHCO). Anal. found: C, 69.32; H, 5.13; N, 11.39. C₂₇H₂₅FN₄O₄ requires: C, 69.38; H, 5.16; N, 11.47%.

Mannich bases 9-19 were synthesized using same procedure.

1-Piperidinomethyl-3-[4'-(4'-fluorobenzyloxy)-benzohydrazono]isatin, 9

m.p. 166–68°C; yield 78%; IR (KBr): 3460 (NH), 2829 (>N-CH₂-N<), 1668 (CO), 1256 (CH₂O), 1060 cm⁻¹ (C-F); 'H NMR (CDCl₃): δ 1.50–1.55 (m, 6H, -CH₂CH₂-), 2.55–2.59 (t, 4H, >N-CH₂-O-), 4.49 (s, 2H, >N-CH₂-N<), 5.31 (s, 2H, >N-CH₂-O-), 6.90–8.15 (m, 12H, Ar-H), 13.85 (s, 1H, NHCO). Anal. found: C, 69.07; H, 5.52; N, 11.49. C₂₇H₂₅FN₄O₄ requires: C, 69.12; H, 5.59; N, 11.55%.

1-(2-Methylpiperidinomethyl)-3-[4'-(4'-fluorobenzyloxy)-benzohydrazono]isatin, 10

m.p. 134–36°C; yield 56%; IR(KBr): 3460 (NH), 2829 (>N-CH₂-N<), 1668 (CO), 1256 (CH₂O), 1055 cm⁻¹ (C-F); 'H NMR (CDCl₃): δ 1.21 (d, 3H, CHMe), 1.40–1.45 (m, 6H, -CH₂CH₂-), 2.40–2.51 (m, 3H, -CH₂-N-CH₂-), 4.49 (s, 2H, >N-CH₂-N<), 5.31 (s, 2H, >N-CH₂-O-), 6.90–8.15 (m, 12H, Ar-H), 13.85 (s, 1H, NHCO). Anal. found: C, 69.51; H, 5.78; N, 11.09. C₂₇H₂₅FN₄O₄ requires: C, 69.58; H, 5.84; N, 11.19%.

1-Pyrrolidinomethyl-3-[4'-(4'-fluorobenzyloxy)-benzohydrazono]isatin, 11

m.p. 166–68°C; yield 77%; IR(KBr): 3489 (NH), 2819 (>N-CH₂-N<), 1684 (CO), 1251 (CH₂O), 1050 cm⁻¹ (C-F); 'H NMR (CDCl₃): δ 1.31–1.36 (m, 4H, -CH₂-), 2.35–2.39 (t, 4H, >N-CH₂-N<), 4.52 (s, 2H, >N-CH₂-N<), 5.30 (s, 2H, >N-CH₂-O-), 7.00–8.11 (m, 12H, Ar-H), 13.76 (s, 1H, NHCO). Anal. found: C, 68.57; H, 5.28; N, 11.83. C₂₇H₂₅FN₄O₄ requires: C, 68.63; H, 5.33; N, 11.86%.
1-N-Methylpiperazinomethyl-3-[4’-(4’)-fluorobenzoxy]-benzohydrazono[isatin], 12

m.p. 198–200°C, yield 70%; 1H NMR (CDCl3): δ 1.85 (s, 3H, N-Me), 2.35–2.41 (t, 4H, -CH2-N-CH2-), 2.55–2.58 (t, 4H, -CH2(NMe)CH2-), 4.55 (s, 2H, >N-CH2-N<), 5.33 (s, 2H, >N-CH2-N<), 7.05–7.99 (m, 12H, Ar-H), 13.98 (s, 1H, NHCO). Anal. found: C, 70.27; H, 5.32; N, 12.40.

C27H27FN4O3 requires: C, 70.32; H, 5.36; N, 12.43%.

1-Ethylpiperazinomethyl-3-[4’-(4’)-fluorobenzoxy]-benzohydrazono[isatin], 13

m.p. 150°C, yield 67%; 1H NMR (CDCl3): δ 1.06–1.11 (t, 3H, -CH2CH3), 1.65–1.71 (t, 4H, -CH2-N-CH2-), 2.35–2.41 (q, 2H, -CH2CH2-), 2.47–2.61 (t, 4H, -CH2N(Ph)CH2-), 4.54 (s, 2H, >N-CH2-N<), 5.33 (s, 2H, >N-CH2-N<), 7.05–8.19 (m, 12H, Ar-H), 13.85 (s, 1H, NHCO). Anal. found: C, 76.50; H, 5.82; N, 13.55. C29H31FN4O3 requires: C, 76.56; H, 5.86; N, 13.58%.

1-N-Phenylpiperazinomethyl-3-[4’-(4’)-fluorobenzoxy]-benzohydrazono[isatin], 14

m.p. 182–84°C; yield 50%; IR(KBr): 3439 (NH), 2829 (>N-CH2-N<), 1674 (CO), 1250 (>CH=O), 1057 cm−1 (C-F); 1H NMR (CDCl3): δ 2.30–2.38 (t, 4H, -CH2-N-CH2-), 2.46–2.58 (t, 4H, -CH2-N(Ph)CH2-), 4.54 (s, 2H, >N-CH2-N<), 5.37 (s, 2H, >N-CH2-O<), 7.00–8.20 (m, 17H, Ar-H), 13.85 (s, 1H, NHCO). Anal. found: C, 70.27; H, 5.32; N, 12.40. C23H29FN4O3 requires: C, 70.32; H, 5.36; N, 12.43%.

1-N-Benzylpiperazinomethyl-3-[4’-(4’)-fluorobenzoxy]-benzohydrazono[isatin], 15

m.p. 164°C; yield 62%; 1H NMR (CDCl3): δ 2.39–2.48 (t, 4H, -CH2-N-CH2-), 2.66–2.71 (t, 4H, -CH2-N(CH2Ph)CH2-), 3.28 (s, 2H, -CH2CH2-), 4.54 (s, 2H, >N-CH2-N<), 5.27 (s, 2H, >N-CH2-O<), 7.09–8.22 (m, 17H, Ar-H), 13.95 (s, 1H, NHCO). Anal. found: C, 70.65; H, 5.54; N, 12.02. C31H31FN4O3 requires: C, 70.69; H, 5.58; N, 12.12%.

1-Dimethylaminomethyl-3-[4’-(4’)-fluorobenzoxy]benzohydrazono[isatin], 16

m. p. 188–90°C; yield 72%; IR (cm−1): 3438 (NH), 2836 (>N-CH2-N<), 1696 (CO), 1235 (>CH=O), 1060 cm−1 (C-F); 1H NMR (CDCl3): δ 2.31 (s, 6H, NMe2), 4.30 (s, 2H, >N-CH2-N<), 5.32 (s, 2H, >N-CH2-O<), 7.02–8.15 (m, 12H, Ar-H), 13.98 (s, 1H, CONH). Anal. found: C, 71.27; H, 5.15; N, 12.49. C21H27FN4O3 requires: C, 71.25; H, 5.19; N, 12.55%.

1-Diethylaminomethyl-3-[4’-(4’)-fluorobenzoxy]benzohydrazono[isatin], 17

m. p. 186–88°C; yield 70%; IR (cm−1): 3431 (NH), 2834 (>N-CH2-N<), 1688 (CO), 1235 (>CH=O), 1055cm−1 (C-F); 1H NMR (CDCl3): δ 1.87–2.00 (t, 6H, CH2Me), 2.13–2.20 (q, 4H, CH2Me), 4.41 (s, 2H, >N-CH2-N<), 5.39 (s, 2H, >N-CH2-O<), 7.06–8.21 (m, 12H, Ar-H), 13.78 (s, 1H, CONH). Anal. found: C, 68.27; H, 5.71; N, 11.79. C25H27FN4O3 requires: C, 68.34; H, 5.74; N, 11.81%.

1-Di-n-propylaminomethyl-3-[4’-(4’)-fluorobenzoxy]benzohydrazono[isatin], 18

m. p. 170(d)°C yield 65%; 1H NMR (CDCl3): δ 1.18–1.28 (t, 6H, CH2CH3), 1.52–1.60 (m, 4H, CH2CH2Me), 2.17–2.22 (t, 4H, CH2CH2Me), 4.40 (s, 2H, >N-CH2-N<), 5.36 (s, 2H, >N-CH2-O<), 7.11–8.11 (m, 12H, Ar-H), 13.88 (s, 1H, CONH). Anal. found: C, 69.25; H, 6.18; N, 11.06. C29H31FN4O3 requires: C, 69.30; H, 6.22; N, 11.15%.

ANTIFUNGAL ACTIVITY

All the compounds 3-19 were screened for their in vitro antifungal potential against human pathogenic fungi, namely Candida albicans, Cryptococcus neoformans, Candida parapsilosis, Trichophyton mentagrophytes and Aspergillus fumigatus using tube dilution method[13] at a maximum concentration of 100 μg/mL in DMSO. The spore suspension of 10⁵ spores/mL was used for this purpose. The drug dilutions were made serially. The test was performed at 29°C and MIC in μg/mL was recorded by visual observation after 24–72 h incubation. Suitable controls: Broth control (without infection), growth control (with infection), solvent DMSO, drug controls (all test compounds), and fluconazole (as standard drug) were set under identical conditions. The last tube with no apparent growth of organism represented the MIC of compounds. Antifungal activity data are presented in Table 1.

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REFERENCES


