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SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL POTENTIAL OF 1-AMINOMETHYL-3-{4′-(4″-NITROBENZYLOXY) -BENZOYLHYDRAZONO} -5-METHYLINDOLIN-2-ONES

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ABSTRACT

A new series of 1-aminomethyl- 3-{4′-(4″-nitrobenzyloxy)benzoylhydrazono}-5-methylindolin-2-ones (Mannich bases) have been synthesized and screened for their antifungal potential against human pathogenic fungi. The structures of the compounds have been established with the help of elemental analysis and spectral data (IR, ^{1}H NMR and Mass).

Keywords: Indolin-2,3-dione, Schiff base, Mannich base, aminomethylation, antifungal activity.

Introduction

Indolin-2,3-diones and their derivatives possess wide variety of biological activities viz., anticonvulsant[1], antitubercular[2], cytotoxic[3,] antimicrobial[4,5], antileishmanial[6], antifertility[7], analgesic[8], antiinflammatory[9] and enzyme inhibitory[10]. Recently number of articles[11-26] have been published on the chemistry, biological potential and toxicity of indolin-2, 3-diones. In the light of biological activity profile of indolin-2, 3-diones and in continuation of our work[27-30] on biologically active heterocycles, it was considered of interest to synthesize a new series of 1-aminomethyl -3-{4′-(4″-nitrobenzyloxy)benzoylhydrazono}- 5-methylindolin-2-ones (Mannich bases).

4-(4′-Nitrobenzyloxy)-benzoylhydrazine **2** was prepared by hydrazinolysis of methyl 4-(4′-nitrobenzyloxy)-benzoate **1** which in turn was obtained by O-benzylation of methyl 4-hydroxybenzoate with 4-nitrobenzyl bromide. Benzoylhydrazine **2** on condensation with 5-methylindolin-2, 3-diones in equimolar proportion, gave 3-{4′-(4″-nitrobenzyloxy)-

benzoylhydrazono}-5-methylindolin-2-ones (Schiff bases) **3-8**. Compound **3** on being subjected to aminomethylation with secondary amines in the presence of formaldehyde, gave 1-aminomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin-2-ones (Mannich bases) **9-17 (Scheme I)**.

Antifungal activity

All the compounds **3-17** were screened for their *in-vitro* antifungal potential against human pathogenic fungi *Candida albicans* (CA), *Cryptococcus neoformans* (CN), *Trichophyton mentagrophytes* (TM) and *Aspergillus fumigatus* (AF) using tube dilution method[31,32] at a maximum concentration of 100 µg/mL in DMSO. The spore suspension of 10^5 spores/mL was used for this purpose. The drug dilutions were made serially. The test was performed at 29^{o}C and Minimum Inhibitory Concentration (MIC) in µg/mL was recorded by visual observation after 24-72 h incubation. Suitable controls: broath control (without infection), growth control (with infection), solvent DMSO, drug controls (all test compounds) and ketoconazole (as standard drug) were set under identical conditions. The last tube with no apparent growth of organism represented the MIC of compound. Antifungal activity data are presented in **Table I.**

Table 1

Minimum Inhibitory Concentration, MIC (µg/mL) of compounds against Fungi

Compd	*Candida albicans (CA)*	*Cryptococcus neoformans (CN)*	*Trichophyton mentagrophytes(TM)*	*Aspergillus fumigatus (AF)*
3	>100	>100	>100	>100
4	25	25	6.25	12.5
5	50	6.25	12.5	12.5
6	25	3.12	6.25	12.5
7	>100	>100	12.5	12.5
8	50	>100	12.5	12.5
9	>100	>100	25	25
10	12.5	50	6.25	12.5
11	12.5	50	6.25	12.5
12	>100	25	25	25
13	>100	>100	50	25
14	>100	>100	12.5	12.5
15	>100	>100	25	12.5
16	50	25	12.5	50
17	50	12.5	12.5	50
Ketokonazole (Standard drug)	0.39	0.78	3.12	6.25

Experimental

The melting points were determined in open capillary tubes in sulphuric 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 δ (ppm). Mass spectra were recorded on Shimadzu GC-MS Q P2010 instrument. 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 vapours.

Methyl 4-(4′-nitrobenzyloxy)-benzoate, 1

A mixture of methyl 4-hydroxybenzoate (0.045 mol), 4-nitrobenzyl bromide (0.045 mol) and anhyd. K_2CO_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. Yield 78%, m.p.182-84°C; IR (KBr): 1708 (CO), 1529, 1350 (NO_2), 1261 cm^{-1}(-CH_2O-).

4-(4′-Nitrobenzyloxy)-benzoylhydrazine, 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 and the contents were poured into water. The solid thus obtained was filtered, washed with water, dried and purified by recrystallization from 1-propanol. Yield 84%, m.p. 202-04°C; IR (KBr): 3315, 3179 ($NHNH_2$), 1659 (CONH), 1527, 1350 (NO_2), 1255 cm^{-1} (-CH_2O-).

3-{4′-(4″-Nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 3

A mixture of 4-(4'-nitrobenzyloxy)-benzoylhydrazine **2** (0.01 mol) and 5-methylindolin-2,3-dione (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. Yield 76%, m.p.>280°C; IR (cm-^{1}): 3480, 3170 (NH), 1691(CO), 1528, 1345 (NO_2), 1254 (-CH_2O-); MS (m/z): 430 ($M^{+\cdot}$). (Found: C, 64.10; H, 4.10; N, 12.96.Calcd. for $C_{23}H_{18}N_4O_5$: C, 64.18; H, 4.22; N, 13.02%).

Compounds **4-8** were synthesised by similar methods using 1–Methyl, ethyl, benzyl, acetyl and hydroxymethyl-5-methylindolin-2, 3-diones.

1-Methyl -3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 4

Yield 86%; m.p.260 (d)°C; ^{1}H NMR (DMSO-d_6) δppm: 2.54 (3H, s, ArMe), 2.63 (3H, s, NMe), 5.25 (2H, s -CH_2O-), 6.96-8.02 (11H, m, Ar-H), 13.98 (1H, s, CONH), MS m/z: 444 ($M^{+\cdot}$). (Found: C, 64.76; H, 4.43; N, 12.66. Calcd. for $C_{24}H_{20}N_4O_5$: C, 64.86; H, 4.50; N, 12.61%).

1-Ethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 5

Yield 73 %; m.p 200-02°C; ^{1}H NMR (DMSO-d_6) δppm: 1.12-1.19 (3H, t, NCH_2CH_3), 2.56 (3H, s, ArMe), 2.86-3.13 (2H, q, NCH_2CH_3), 5.25 (2H, s, -CH_2O-), 6.88-8.12 (11H, m, Ar-H), 13.98 (1H, s, CONH), MS m/z: 458 ($M^{+\cdot}$). (Found: C, 65.37; H, 4.77; N, 12.17. Calcd. for $C_{25}H_{22}N_4O_5$: C, 65.49; H, 4.84; N, 12.22%).

1-Benzyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 6

Yield 67 %; m.p 218-20°C; ^{1}H NMR (DMSO-d_6) δppm: 2.56 (3H, s, ArMe), 4.92(2H, s, NCH_2Ph), 5.25 (2H, s, -CH_2O-), 6.96-8.11 (16H, m, Ar-H), 13.90 (1H, s, CONH), MS m/z: 520 ($M^{+\cdot}$). (Found: C, 69.17; H, 4.60; N, 10.67. Calcd. for $C_{30}H_{24}N_4O_5$: C, 69.22; H, 4.65; N, 10.76%).

1-Acetyl -3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 7

Yield 66 %; m.p.220°C; ^{1}H NMR (DMSO-d_6) δppm: 2.32 (3H, s, COMe), 2.56 (3H, s, ArMe), 5.25 (2H, s, -CH_2O-), 6.91-8.02 (10H, m, Ar-H), 13.89 (1H, s, CONH), MS m/z: 472 ($M^{+\cdot}$) (Found: C, 63.52; H, 4.23; N, 11.83. Calcd. for $C_{25}H_{20}N_4O_6$: C, 63.56; H, 4.27; N, 11.86%);

1-Hydroxymethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 8

Yield 70 %; m.p >260°C; ^{1}H NMR (DMSO-d_6) δppm: 2.60 (3H, s, ArMe), 5.10 (2H, s, NCH_2OH), 5.25 (2H, s, -CH_2O-), 6.42 (1H, bs, NCH_2OH), 6.94-8.00 (10H, m, Ar-H), 13.87 (1H, s, CONH), MS m/z: 460 ($M^{+\cdot}$). (Found: C, 62.57; H, 4.35; N, 12.13. Calcd. for $C_{24}H_{20}N_4O_6$: C, 62.60; H, 4.38; N, 12.17%).

1-Morpholinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 9

To a suspension of **3** (0.005 mol) in DMF, formaldehyde (0.5mL, 37% aq. solution) and morpholine (0.005 mol) were added with vigorous stirring, and the reaction mixture was

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). Yield 74 %, m.p.212-14^{0}C; IR (cm-^{1}): 3480 (NH), 2804 (>N-CH_2-N<), 1683 (CO), 1529, 1349 (NO_2), 1253 (-CH_2O-) 1157 (-CH_2-O-CH_2-); ^{1}H NMR ($CDCl_3$) δppm: 2.60 (3H, s, ArMe), 2.63-2.67 (4H, t, -CH_2-N-CH_2-), 3.69-3.72 (4H, t, -CH_2-O-CH_2-), 4.52 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 6.77-8.12 (11H, m, Ar-H), 13.77 (1H, s, CONH); MS m/z: 529($M^{+\cdot}$). (Found: C, 63.44; H, 5.04; N, 13.17. Calcd. for $C_{28}H_{27}N_5O_6$: C, 63.51; H, 5.14; N, 13.23 %).

1-Piperidinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 10

Yield 68 %, m.p.212-14^{0}C; IR (cm-^{1}): 3445 (NH), 2850 (>N-CH_2-N<), 1686 (CO), 1521, 1349 (NO_2), 1250 (-CH_2O-); ^{1}H NMR ($CDCl_3$): 1.42-1.59 (6H, m,-$CH_2CH_2CH_2$-), 2.56 (3H, s, ArMe), 2.58-2.61 (4H, t, -CH_2-N-CH_2-), 4.52(2H, s, >N-CH_2-N<), 5.25 (2H, s,-CH_2O-), 7.07-8.01 (11H, m, Ar-H), 13.87 (1H, s, CONH); MS m/z: 527 ($M^{+\cdot}$). (Found: C, 65.92; H, 5.46; N, 13.18. Calcd. for $C_{29}H_{29}N_5O_5$: C, 66.02; H, 5.54; N, 13.27 %).

1-Pyrrolidinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin-2-one, 11

Yield 68 %, m.p.200^{0}C; IR (cm-^{1}): 3440 (NH), 2843 (>N-CH_2-N<), 1678 (CO), 1522, 1345 (NO_2), 1251 (-CH_2O-); ^{1}H NMR ($CDCl_3$): 1.35-1.42 (4H, m, -CH_2CH_2-), 2.33-2.46 (4H, t, -CH_2-N-CH_2-), 2.59 (3H, s, ArMe), 4.54 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 7.05-8.15 (11H, m, Ar-H), 13.83 (1H, s, CONH); MS m/z: 513 ($M^{+\cdot}$). (Found: C, 65.40; H, 5.26; N, 13.57. Calcd. for $C_{28}H_{27}N_5O_5$: C, 65.49; H, 5.30; N, 13.64 %).

1-N-Methylpiperazinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindo-

lin-2-one, 12

Yield 69 %, m.p.218(d) ^{0}C; ^{1}H NMR ($CDCl_3$): 1.87 (3H, s, N-Me), 2.33-2.39 (4H, t, -CH_2-N-CH_2-), 2.53 (3H, s, ArMe), 2.56-2.65 (4H, t, -CH_2-N(Me)-CH_2-), 4.55 (2H, s, >N-CH_2-N<), 5.25 (2H, s,-CH_2O-), 6.88-7.88 (11H, m, Ar-H), 14.00 (1H, s, CONH); MS m/z: 542 ($M^{+\cdot}$). (Found: C, 64.08; H, 5.49; N, 15.47. Calcd. for $C_{29}H_{30}N_6O_5$: C, 64.19; H, 5.57; N, 15.49 %).

1-N-Ethylpiperazinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin-2-one, 13

Yield 69 %, m.p.218(d) ^{0}C; ^{1}H NMR ($CDCl_3$): 1.87-1.92 (3H, t, -CH_2Me), 2.00-2.09 (2H, q, -CH_2Me), 2.33-2.39 (4H, t, -CH_2-N-CH_2-), 2.54 (3H, s, ArMe), 2.59-2.67 (4H, t, -CH_2-N(Et)-CH_2-), 4.58 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 6.88-7.99 (11H, m, Ar-H), 13.89 (1H, s, CONH); MS m/z: 556 ($M^{+\cdot}$). (Found: C, 64.78; H, 5.68; N, 15.07. Calcd. for $C_{30}H_{32}N_6O_5$: C, 64.73; H, 5.79; N, 15.10 %).

1-N-Phenylpiperazinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin-2-one, 14

Yield 60 %, m.p.214-16^{0}C (d); IR (cm-^{1}): 3453 (NH), 2839 (>N-CH_2-N<), 1680 (CO), 1529, 1346 (NO_2), 1244(-CH_2O-); ^{1}H NMR ($CDCl_3$): 2.33-2.41(4H, t, -CH_2-N-CH_2-),2.56 (3H, s, ArMe), 2.60-2.69 (4H, t, -CH_2-N(Ph)-CH_2-), 4.55 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 7.04-8.28 (16H, m, Ar-H), 13.90 (1H, s, CONH); MS m/z: 604 ($M^{+\cdot}$). (Found: C, 67.52; H, 5.26; N, 13.87. Calcd. for $C_{34}H_{32}N_6O_5$: C, 67.54; H, 5.33; N, 13.90 %).

1-N-Benzylpiperazinomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 15

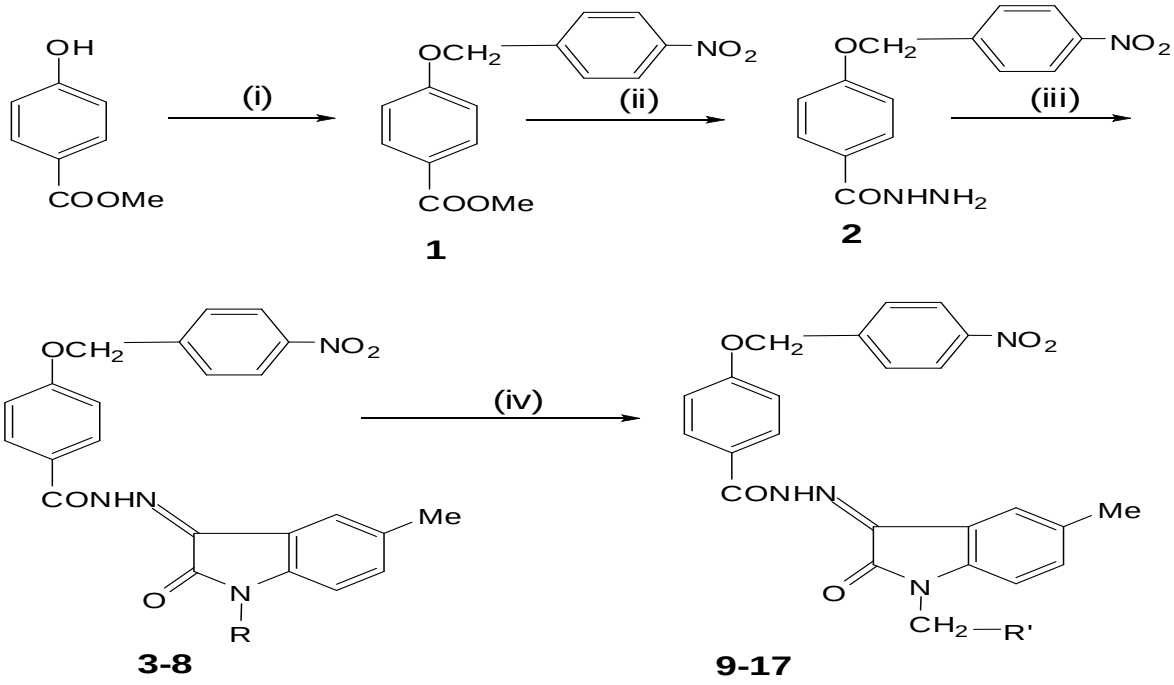
Yield 55 %, m.p.186-88^0 C; ^{1}H NMR ($CDCl_3$): 2.31-2.41 (4H, t, -CH_2-N-CH_2-), 2.56 (3H, s, ArMe), 2.62-2.71 (4H, t, -CH_2-N(CH_2Ph)-CH_2-), 4.49 (2H, s, CH_2Ph), 4.55 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 7.04-8.26 (16H, m, Ar-H), 13.93 (1H, s, CONH); MS m/z: 618 ($M^{+\cdot}$). (Found: C, 67.82; H, 5.46; N, 13.53. Calcd. for $C_{35}H_{34}N_6O_5$: C, 67.95; H, 5.54; N, 13.58 %).

1-Dimethylaminomethyl-3-{4′-(4″-nitrobenzyloxy)-benzoylhydrazono}-5-methylindolin -2-one, 16

Yield 48 %, m.p.140 ^{0}C; IR (cm-^{1}): 3427 (NH), 2832 (>N-CH_2-N<), 1699 (CO), 1523, 1348 (NO_2), 1258 (-CH_2O-); ^{1}H NMR ($CDCl_3$): 2.33 (6H, s, NMe_2), 2.63 (3H, s, ArMe), 4.27 (2H, s, >N-CH_2-N<), 5.26 (2H, s, -CH_2O-), 7.00-8.26 (11H, m, Ar-H), 13.98 (1H, s, CONH); MS m/z: 487 ($M^{+\cdot}$). (Found: C, 64.00; H, 5.09; N, 14.30. Calcd. for $C_{26}H_{25}N_5O_5$: C, 64.06; H, 5.17; N, 14.37 %).

1-Diethylaminomethyl-3-[4′-(4″-nitrobenzyloxy)-benzoylhydrazono]-5-methylindolin-2-one, 17

Yield 48 %, m.p.180^{0}C; IR (cm-^{1}): 3433 (NH), 2830 (>N-CH_2-N<), 1692 (CO), 1523, 1345 (NO_2), 1258 (-CH_2O-); ^{1}H NMR ($CDCl_3$): 1.87-2.09 (6H, t, CH_2Me), 2.19-2.28 (4H, q, CH_2Me), 2.56 (3H, s, ArMe), 4.47 (2H, s, >N-CH_2-N<), 5.25 (2H, s, -CH_2O-), 7.00-8.29 (10H, m, Ar-H), 13.98 (1H, s, CONH); MS m/z: 515 ($M^{+\cdot}$). (Found: C, 63.15; H, 5.76; N, 13.50. Calcd. for $C_{28}H_{29}N_5O_5$: C, 63.23; H, 5.67; N, 13.58 %).



(i) 4-Nitrobenzyl bromide
K_2CO_3(anhyd); Me_2CO
(ii) $N_2H_4.H_2O$; 1-Propanol
(iii) 5-Methylindolin-2,3-diones, gl. AcOH; EtOH
(iv) Amines,CH_2O; DMF

R = H, Me, Et, Bz, $COCH_3$, CH_2OH
R′ = morpholino, piperidino, pyrrolidino N-methylpiperazino, N-ethylpiperazino, N-phenylpiperazino, N-benzylpiperazino, dimethylamino,diethylamino

Scheme I

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