**Supplementary File: S1**

# Title: Prevention of testicular damage by indole derivative MMINA via upregulated StAR and CatSper channels with coincident suppression of oxidative stress and inflammation: *In silico* and *in Vivo* validation

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## Supplementary: Biological Characterization of MMINA

*Synthesis of 2-(6-Methoxy-2-methyl-1H-indol-3-yl) acetohydrazide* **(1)**

The methyl ester of indomethacin (0.01 mol) and hydrazine hydrate (99%) (0.2 mol) in presence of absolute ethanol (50 mL) were refluxed for 30 hours. The reaction mixture was concentrated by using rota vapor and poured in a beaker containing crushed ice in small portions while stirring and kept for 4 hours at room temperature. The solid was separated out by filtration. The product was dried and recrystallized from ethanol. The product was carefully checked by thin layer chromatography. The first compound was 2-(6-methoxy-2-methyl-1H-indol-3-yl) acetohydrazide compound **(1)**, and was obtained as the major product. The second compound, 4-chlorobenzohydrazide **(2)** was obtained as minor product. Both the compounds were fully characterized by the spectral data.

2-(6-Methoxy-2-methyl-1*H*-indol-3-yl) acetohydrazide (**1**). Color: white; Yield: 70%; m.p.: 168–170 °C; UV $\lambda$max (Methanol) = 280 nm; $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.38 (3H, s, $CH_3$), 3.54 (2H, s, $CH_2$), 3.80 (3H, s, $OCH_3$), 4.26 (2H, s, $NH_2$, $D_2O$ exchg.), 6.67 (1H, d, *J* = 8.5 Hz, Ar-H), 7.16 (2H, d, *J* = 7.5 Hz, Ar-H), 9.16 (1H, s, NH, $D_2O$ exchg.), 10.62 (1H, s, CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.0 ($CH_3$), 30.2 ($CH_2$), 55.8 ($OCH_3$), 101.1, 105.1, 109.8, 110.0, 111.7, 128.0, 129.3, 129.7, 130.6, 134.3, 153.4, 170.8 (C=O); MS: *m*/*z* = 233.11 $[M]^+$, 234.07 $[M+1]^+$; Analysis: $C_{12}H_{15}N_3O_2$ for, calcd. C 61.79, H 6.48, N 18.01 %; found C 61.58, H 6.46, N 18.05 %.

4-Chlorobenzohydrazide **(2)**. Color: white; Yield: 20%; M.p.: 148–150 °C; UV $\lambda$max (Methanol) = 230 nm; $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 4.53 (2H, s, $NH_2$, $D_2O$ exchg.), 7.52 (2H, d, *J* = 8.5 Hz, Ar-H), 7.84 (2H, d, *J* = 8.5 Hz, Ar-H), 9.87 (1H, s, CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 128.86, 129.32, 132.50, 136.25, 165.29; MS: *m*/*z* = 170.45 $[M]^+$; Analysis: $C_7H_7N_2OCl$ for, calcd. C 49.28, H 4.14, N 16.42%; found C 49.37, H 4.12, N 16.46%.

3.3. *General procedure for the synthesis of 2-(5-methoxy-2-methyl-1-indol-3yl)-N-[(E)-substituted phenyl methylidine] aceto hydrazide derivatives* **(S1-S18)**. A solution of indole hydrazide **(1)** (371 mg, 1.0 mmol) in EtOH (15 mL) containing an appropriate substituted benzaldehyde (1.1 mmol) and a catalytic amount of Glacial acetic acid was heated under reflux for 3 hours. After cooling, 5 mL of water was added to the mixture and kept in a refrigerator for 12 hours. The product was obtained by filtration. The compound was washed several times with cold water. The compound obtained was recrystallized from ethanol.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-phenylmethylidene] acetohydrazide* **(S1):** Yield: 70%; m.p.: 170–172 °C; IR (KBr) $cm^{-1}$: 3412 (NH), 3024 (C-H), 1654 (C=O), 1637 (C=N); $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.55 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.65-8.00 (8H, m, Ar-H), 10.62 (1H, s, =CH), 11.26 (1H, s, -NH, $D_2O$ exchg.), 11.9 (1H, s, CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.2 ($CH_3$), 28.2 ($CH_2$), 55.5 ($OCH_3$), 100.7, 104.7, 110.0, 111.3, 127.2, 127.4, 127.6, 129.0, 129.2, 129.3, 130.0, 134.3, 134.8,

153.3, 167.7 (C=O); MS: *m*/*z* = 321.37 [M]$^+$; Analysis: for $C_{19}H_{19}N_3O_2$, calcd. C 71.01, H 5.96, N 13.08 %; found C 71.25, H 5.94, N 13.11%.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(4-nitrophenyl)methylidene] acetohydrazide* **(S2):** Yield: 75%; m.p.: 220–222 °C; IR (KBr) cm$^{-1}$: 3411 (NH), 3000 (C-H), 1654 (C=O), 1617 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.58 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.97-8.26 (7H, m, Ar-H), 10.63 (1H, s, =CH), 11.70 (1H, s, -NH, $D_2O$ exchg.), 12.2 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.6 ($CH_3$), 28.2 ($CH_2$), 55.0 ($OCH_3$), 100.2, 110.8, 123.9, 124.0, 127.6, 128.0, 128.6, 129.6, 140.4, 142.0, 143.0, 144.0, 145.5, 151.0, 163.0, 175.0; MS: *m*/*z* = 366.37 [M]$^+$; Analysis: for $C_{19}H_{18}N_4O_4$, calcd. C 62.29, H 4.95, N 15.29 %; found C 62.14, H 4.97, N 15.25 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(3-nitrophenyl)methylidene] acetohydrazide* **(S3):** Yield: 68%; m.p.: 200–202°C; IR (KBr) cm$^{-1}$: 3412 (NH), 3237 (C-H), 1654 (C=O), 1617 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.58 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.99-8.57 (7H, m, Ar-H), 10.63 (1H, s, =CH), 11.5 (1H, s, NH, $D_2O$ exchg.), 12.18 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.5 ($CH_3$), 27.9 ($CH_2$), 55.0, 100.2, 103.9, 109.4, 123.8, 124.2, 128.5, 130.3, 131.7, 133.3, 136.0, 140.33, 145.5, 148.1, 152.8, 162.2, 170.0; MS: *m*/*z* = 366.37 [M]+; Analysis: for $C_{19}H_{18}N_4O_4$, calcd. C 62.29, H 4.95, N 15.29 %; found C 62.36, H 4.93, N 15.24 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2-nitrophenyl)methylidene] acetohydrazide* **(S4):** Yield: 70%; m.p.: 210–212°C; IR (KBr) cm$^{-1}$: 3407 (NH), 3063 (C-H), 1654 (C=O), 1617 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.58 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.98-8.25 (7H, m, Ar-H), 10.63 (1H, s, =CH), 11.90 (1H, s, -NH, $D_2O$ exchg.), 12.10 (1H, s, -CONH, D2O exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.60 ($CH_3$), 27.8 ($CH_2$), 54.9 ($OCH_3$), 100.0, 103.8, 109.4, 110.6, 124.5, 127.8, 129.6, 133.4, 134.0, 141.4, 143.2, 147.9, 148.2, 152.0; 167.0, 170.0; MS: *m*/*z* = 366.37 [M]+; Analysis: for $C_{19}H_{18}N_4O_4$, calcd. C 62.29, H 4.95, N 15.29 %; found C 62.15, H 4.97, N 15.24 %

*N'-[(E)-(4-chlorophenyl)methylidene]-2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide* **(S5):** Yield: 80%; m.p.: 180–182 °C; IR (KBr) cm$^{-1}$: 3411 (NH), 3071 (C-H), 1654 (C=O), 1597 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.36 (3H, s, -$CH_3$), 3.55 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.60-8.45 (7H, m, Ar-H), 10.63 (1H, s, =CH), 11.3 (1H, s, NH, $D_2O$ exchg.), 12.00 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.6 ($CH_3$), 28.2 ($CH_2$), 55.1 ($OCH_3$), 101.0, 128.5, 128.7, 128.8, 128.9, 129.5, 136.6, 146.7, 148.0, 149.0, 150.0, 151.0, 152.1,162.7, 172.0; MS: *m*/*z* = 355.81 [M]$^+$; Analysis: for $C_{19}H_{18}N_3O_2Cl$, calcd. C 64.13, H 5.10, N 11.81 %; found C 64.33, H 5.12, N 11.83 %.

*N'-[(E)-(2,4-dichlorophenyl)methylidene]-2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide* **(S6)**: Yield: 65%; m.p.: 238–240 °C; IR (KBr) cm$^{-1}$: 3411 (NH), 2940 (C-H), 1654 (C=O), 1617 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.36 (3H, s, -$CH_3$), 3.59 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.59-8.61 (6H, m, Ar-H), 10.62 (1H, s, =CH), 11.51 (1H, s, -NH, $D_2O$ exchg.); 11.7 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.5 ($CH_3$), 27.8 ($CH_2$), 55.0 ($OCH_3$), 100.2, 103.7, 109.4, 110.8, 127.8, 128.7, 129.2, 130.0, 133.4, 133.6, 134.0, 137.5, 140.9, 154.0, 168.0, 172.0S; MS: *m*/*z* = 390.26 [M]$^+$; Analysis: for $C_{19}H_{17}N_3O_2Cl_2$, calcd. C 58.47, H 4.35, N 10.77 %; found C 58.25, H 4.33, N 10.74 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(3,4-dimethoxyphenyl)methylidene] acetohydrazide* **(S7):** Yield: 70%; m.p.: 210–212°C; IR (KBr) cm$^{-1}$: 3299 (NH), 3011 (C-H), 1654 (C=O), 1599 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.40 (3H, s, -$CH_3$), 3.70 (2H, s, $CH_2$), 3.84 (3H, s, -$OCH_3$), 6.50-8.40 (6H, m, Ar-H), 10.50 (1H, s, =CH), 11.20 (1H, s, -NH, $D_2O$ exchg.), 11.5 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 12.2 ($CH_2$), 55.6 ($OCH_3$), 55.8 ($OCH_3$), 56.0 ($OCH_3$), 101.0, 121.4, 122.0, 127.5, 143.1, 151.0, 175.0; MS: *m*/*z* = 381.42 [M]$^+$; Analysis: for $C_{21}H_{23}N_3O_4$, calcd. C 66.13, H 6.08, N 11.02 %; found C 66.31, H 6.10, N 11.05 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2-methoxyphenyl)methylidene] acetohydrazide* **(S8)**: Yield: 60%; m.p.: 220–222°C; IR (KBr) cm$^{-1}$: 3315 (NH), 3017 (C-H), 1664 (C=O), 1601 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.36 (3H, s, -$CH_3$), 3.56 (2H, s, $CH_2$), 3.87 (3H, s, -$OCH_3$), 7.00-8.82 (11H, m, Ar-H), 10.61 (1H, s, =CH), 11.23(1H, s, NH, $D_2O$ exchg.), 11.92 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1

($CH_3$), 28.1 ($CH_2$), 56.1 ($OCH_3$), 63.4 ($OCH_3$), 111.2, 112.2, 112.3, 121.2, 122.7, 125.8, 126.0, 129.0, 130.0, 132.1, 137.0, 144.0, 158.2, 162.3; MS: *m*/*z* = 351.39 $[M]^+$; Analysis: for $C_{20}H_{21}N_3O_3$, calcd. C 68.36, H 6.02, N 11.96 %; found C 68.50, H 6.00, N 11.93 %.

*N'-[(E)-(4-hydroxyphenyl)methylidene]-2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide* **(S9):** Yield: 70%; m.p.: 230–232 °C; IR (KBr) $cm^{-1}$: 3411 (OH), 3411 (NH), 3300 (C-H), 1654 (C=O), 1609 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.58 (2H, s, $CH_2$), 3.75 (3H, s, -$OCH_3$), 6.59-8.36 (7H, m, Ar-H), 9.88 (1H, s, OH, $D_2O$ exchg.), 10.60 (1H, s, =CH), 11.0 (1H, s, -CONH, $D_2O$ exchg.), 11.73 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.2 ($CH_2$), 55.5 ($OCH_3$), 100.8, 104.9, 109.8, 110.0, 116.1, 128.9, 129.4, 130.5, 134.2, 143.4, 153.3, 159.5, 162.2, 167.4, 170.7, 172.9; MS: *m*/*z* = 337.37 $[M]^+$; Analysis: for $C_{19}H_{19}N_3O_3$, calcd. C 67.64, H 5.68, N 12.46 %; found C 67.43, H 5.70, N 12.43 %.

*N'-[(E)-(3-hydroxyphenyl)methylidene]-2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide* (**S10**): Yield: 60%; m.p.: 145–147 °C; IR (KBr) $cm^{-1}$: 3500 (OH), 3413 (NH), 3023 (C-H), 1654 (C=O), 1617 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.57 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.58-8.17 (7H, m, Ar-H), 9.59 (1H, s, OH, $D_2O$ exchg.), 10.62 (1H, s, =CH), 11.21 (1H, s, -NH, $D_2O$ exchg.), 11.39 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.6 ($CH_3$), 27.6 ($CH_2$), 54.9 ($OCH_3$), 100.1, 104.0, 109.3, 110.8, 112.4, 117.2, 118.2, 128.5, 129.5, 130.0, 133.8, 135.5, 146.2, 152.8, 157.5, 175.0; MS: *m*/*z* = 337.37 [M]+; Analysis: for $C_{19}H_{19}N_3O_3$, calcd. C 67.64, H 5.68, N 12.46 %; found C 67.41, H 5.70, N 12.42 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-{(E)-[4-(dimethylamino) phenyl]methylidene}acetohydrazide* (**S11**): Yield: 65%; m.p.: 200–202 °C; IR (KBr) $cm^{-1}$: 3351 (NH), 2909 (C-H), 1654 (C=O), 1609 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.00 (6H, s, 2×$NCH_3$) 3.59 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 6.59-8.32 (7H, m, Ar-H), 10.60 (1H, s, =CH), 10.97 (1H, s, -NH, $D_2O$ exchg.), 11.62 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.0 ($CH_2$), 30.0 ($NCH_3$), 31.0 ($NCH_3$), 55.5 ($OCH_3$), 100.9, 105.0, 110.0, 111.2, 112.27, 112.3, 122.2, 128.4, 128.7, 128.9, 129.8, 130.5, 143.9, 151.7, 153.3, 172.7; ms: *m*/*z* = 364.44 $[M]^+$; Analysis: for $C_{21}H_{24}N_4O_2$, calcd. C 69.21, H 6.64, N 15.37 %; found C 69.37, H 6.66, N 15.33 %.

*2-(5-methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(3-methoxyphenyl)methylidene] acetohydrazide* (**S12**): Yield: 65%; m.p.: 195–197 °C; IR (KBr) $cm^{-1}$: 3412 (NH), 3000 (C-H), 1654 (C=O), 1636 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.58 (2H, s, $CH_2$), 3.80 (3H, s, -$OCH_3$), 6.59-8.44 (7H, m, Ar-H), 10.67 (1H, s, =CH), 11.28 (1H, s, -NH, $D_2O$ exchg.), 11.46 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.3 ($CH_2$), 55.5 ($OCH_3$), 55.35 ($OCH_3$), 100.8, 104.5, 104.7, 109.8, 110.0, 111.6, 120.3, 129.3, 130.3, 134.3, 134.3, 136.2, 142.9, 146.5, 153.3, 159.9, 173.3; MS: *m*/*z* = 351.39 $[M]^+$; Analysis: for $C_{20}H_{21}N_3O_3$, calcd. C 68.36, H 6.02, N 11.96 %; found C 68.15, H 6.00, N 11.99 %.

*N'-[(E)-(4-ethoxyphenyl)methylidene]-2-(5-methoxy-2-methyl-1H-indol-3-yl)acetohydrazide* (**S13**): Yield: 75%; m.p.: 213–215 °C; IR (KBr) $cm^{-1}$: 3322 (NH), 3042 (C-H), 1654 (C=O), 1571 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 1.33 (3H, t, *J* = 7.5 Hz, $CH_2CH_3$), 2.37 (3H, s, -$CH_3$), 3.57 (2H, s, $CH_2$), 3.74 (3H, s, -$OCH_3$), 4.06 (2H, q, *J* = 7.5 Hz, $OCH_2$), 6.58-8.20 (7H, m, Ar-H), 10.61 (1H, s, =CH), 11.12 (1H, s, -NH, $D_2O$ exchg.), 11.30 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.2 ($CH_3$), 15.0 ($CH_2$), 28.2 ($CH_3$), 55.5 ($OCH_3$), 55.88 ($OCH_2$), 63.7, 100.8, 104.8, 109.8, 111.2, 115.1, 127.2, 129.2, 129.3, 130.5, 134.3, 143.0, 146.5, 153.3, 160.2, 167.5, 173.0; ms: *m*/*z* = 365.42 $[M]^+$; Analysis: for $C_{21}H_{23}N_3O_3$, calcd. C 69.02, H 6.34, N 11.50 %; found C 69.22, H 6.36, N 11.53 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2,4,5-trimethoxyphenyl) methylidene]acetohydrazide* (**S14**): Yield: 60%; m.p.: 238–240 °C; IR (KBr) $cm^{-1}$: 3412 (NH), 2943 (C-H), 1654 (C=O), 1617 (C=N); $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.36 (3H, s, -$CH_3$), 3.51 (2H, s, $CH_2$), 3.78 (12H, s, -4×$OCH_3$), 6.91-8.42 (5H, m, Ar-H), 10.61 (1H, s, =CH), 11.14 (1H, s, -NH, $D_2O$ exchg.), 11.42 (1H, s, -CONH, $D_2O$ exchg.); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 30.1 ($CH_2$), 55.5 ($OCH_3$), 56.4 ($OCH_3$), 60.9 ($OCH_3$), 62.1 ($OCH_3$), 100.8, 104.8, 109.1, 109.8, 110.0, 111.2, 120.8, 130.5, 134.4, 138.9, 142.0, 152.8, 153.3, 155.2, 172.9; ms: *m*/*z* = 411.45 $[M]^+$; Analysis: for $C_{22}H_{25}N_3O_5$, calcd. C 64.22, H 6.12, N 10.21 %; found C 64.35, H 6.14, N 10.24 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2,3,4-trimethoxyphenyl) methylidene]acetohydrazide* (**S15**): Yield: 55%; m.p.: 250–252 °C; IR (KBr) $cm^{-1}$: 3310 (NH), 3048 (C-H), 1654 (C=O), 1595 (C=N); $^1H$ NMR (500 MHz,

DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.59 (2H, s, $CH_2$), 3.84 (12H, s, 4×-$OCH_3$), 6.59-8.74 (5H, m, Ar-H), 10.60 (1H, s, =CH), 11.08 (1H, s, -NH, $D_2O$ exchg.), 11.33 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.5 ($CH_2$), 55.6 ($OCH_3$), 56.2 ($OCH_3$), 56.4 ($OCH_3$), 56.9 ($OCH_3$), 98.3, 101.1, 104.8, 108.4, 109.8, 111.2, 114.1, 129.2, 130.6, 134.4, 138.8, 143.6, 152.1, 153.6, 167.3, 172.9; ms: *m*/*z* = 411.45 [M]+; Analysis: for $C_{22}H_{25}N_3O_5$, calcd. C 64.22, H 6.12, N 10.21 %; found C 64.36, H 6.10, N 10.23 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(3,4,5-trimethoxyphenyl) methylidene]acetohydrazide* (**S16**): Yield: 58%; m.p.: 233–235 °C; IR (KBr) $cm^{-1}$: 3309 (NH), 3015 (C-H), 1654 (C=O), 1577 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.59 (2H, s, $CH_2$), 3.83 (12H, s, 4× -$OCH_3$), 6.97-8.20 (5H, m, Ar-H), 10.61 (1H, s, =CH), 11.28 (1H, s, -NH, $D_2O$ exchg.), 11.40 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.4 ($CH_2$), 55.6 ($OCH_3$), 55.8 ($OCH_3$), 56.3 ($OCH_3$), 60.5 ($OCH_3$), 60.5, 101.1, 104.4, 104.6, 104.7, 109.8, 111.2, 130.3, 130.6, 134.2, 139.3, 142.9, 153.3, 153.6, 173.2; ms: *m*/*z* = 411.45 [M]+; Analysis: for $C_{22}H_{25}N_3O_5$, calcd. C 64.22, H 6.12, N 10.21 %; found C 64.37, H 6.10, N 10.24 %.

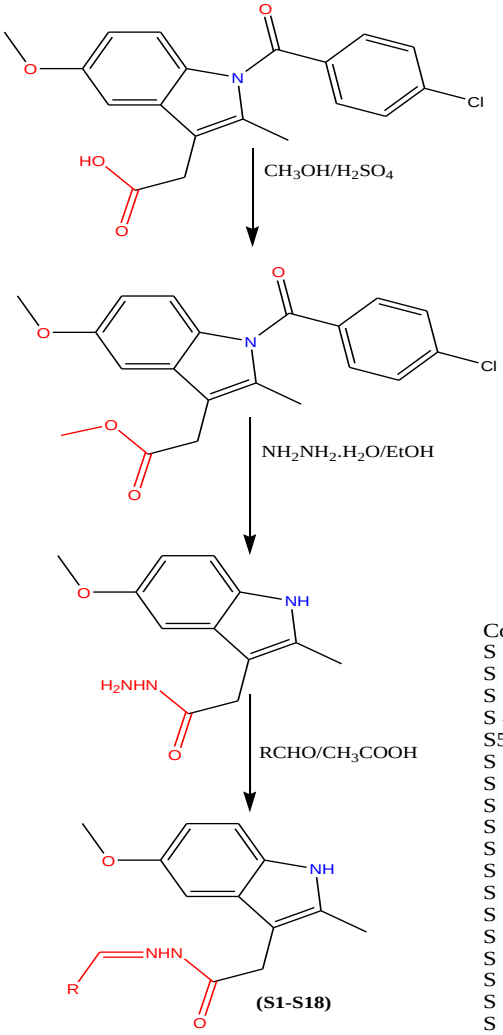
*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2,4,6-trimethoxyphenyl) methylidene]acetohydrazide* (**S17**): Yield: 55%; m.p.: 230–232 °C; IR (KBr) $cm^{-1}$: 3412 (NH), 3056 (C-H), 1654 (C=O), 1612 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.37 (3H, s, -$CH_3$), 3.59 (2H, s, $CH_2$), 3.82 (12H, s, 4× -$OCH_3$), 6.57-8.74 (5H, m, Ar-H), 10.61 (1H, s, =CH), 11.10 (1H, s, NH, $D_2O$ exchg.), 11.80 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 12.1 ($CH_3$), 28.4 ($CH_2$), 55.5 ($OCH_3$), 55.7 ($OCH_3$), 56.3 ($OCH_3$), 60.5 ($OCH_3$), 98.6, 106.7, 111.2, 115.6, 129.9, 142.2, 144.1, 153.3, 159.3, 159.6 162.1, 167.3, 172.9; ms: *m*/*z* = 411.45 [M]+; Analysis: for $C_{22}H_{25}N_3O_5$, calcd. C 64.22, H 6.12, N 10.21 %; found C 64.38, H 6.13, N 10.17 %.

*2-(5-Methoxy-2-methyl-1H-indol-3-yl)-N'-[(E)-(2,4-dimethoxyphenyl) methylidene]acetohydrazide* (**S18**): Yield: 60%; m.p.: 170–172 °C; IR (KBr) $cm^{-1}$: 3413 (NH), 3000 (C-H), 1654 (C=O), 1638 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 2.40 (3H, s, -$CH_3$), 3.60 (2H, s, $CH_2$), 3.82 (3H, s, -$OCH_3$), 6.50-8.70 (6H, m, Ar-H), 10.61 (1H, s, =CH), 11.20 (1H, s, NH, $D_2O$ exchg.); 11.80 (1H, s, -CONH, $D_2O$ exchg.); $^{13}$C NMR (125 MHz, DMSO-$d_6$): $\delta$ = 11.5 ($CH_3$), 28.4 ($CH_2$), 55.0 ($OCH_3$), 55.6 ($OCH_3$), 55.68 ($OCH_3$), 98.2, 104.4, 106.2, 109.5, 110.7, 115.2, 128.8, 130.0, 132.0, 135.8, 138.3, 143.6, 152.8, 158.9, 162.0, 162.4, 164.7, 172.0; ms: *m*/*z* = 381.42 $[M]^+$; Analysis: for $C_{21}H_{23}N_3O_4$, calcd. C 66.13, H 6.08, N 11.02 %; found C 66.34, H 6.10, N 11.05 %.

**Supplementary Table 1: Primers**

| Candidate gene | primer |
|---|---|
| **StAR** | F: 5'-GGG CTC CACCTG CTT CTT G-3'<br>R: 5'- ACC CCT CTG CTC AGG CAT TT −3' |
| **HSD** | F: 5'-GAAGAAGCATGGAGGTCAAC-3'<br>R: 5'-GCAATCAGAGGTTGGGTCAT-3'.′ |
| **CYP20A1** | F: 5′-CCTCGTCCTTTATGCCCTTGGT-3′<br>R: 5′-GTGCCTGAGAATCCAAGTGAGG-3′ |
| **CAT Sper1** | F: 5'-TCGGAGAACCACAGAGAAGAG-3'<br>R: 5'-CACACACCGGGAATATCTTC-3' |
| **CAT Sper2** | F: 5'-TGGCCACAGAGCAGTATTTG-3′<br>R: 5'-TGTCAGGCTGTTGCTTTGTC-3' |
| **iNOs** | F: 5′-CTATGGCCGCTTTGATGTGC-3′<br>R: 5′-CAACCTTGGTGTTGAAGGCG-3′ |

## Scheme1




**(S1-S18)**

| Code | R |
|---|---|
| S 1 | Phenyl |
| S 2 | 4-Nitrophenyl |
| S 3 | 3-Nitrophenyl |
| S 4 | 2-Nitrophenyl |
| S5 | 4-Chlorophenyl |
| S 6 | 2, 4-Dichlorophenyl |
| S 7 | 3,4-Dimethoxyphenyl |
| S 8 | 2-Methoxyphenyl |
| S 9 | 4-Hydroxyphenyl |
| S 10 | 3-Hydroxyphenyl |
| S 11 | 4- Dimethylaminophenyl |
| S 12 | 3-methoxyphenyl |
| S 13 | 4-Ethoxyphenyl |
| S 14 | 2, 4, 5-Trimethoxyphenyl |
| S 15 | 2, 3, 4-Trimethoxyphenyl |
| S 16 | 3, 4, 5-Trimethoxyphenyl |
| S 17 | 2, 4, 6-Trimethoxyphenyl |
| S 18 | 2, 4-Dimethoxyphenyl |