Supporting Information

Design, Synthesis and Biological Profiling of Aryl Piperazine Based Scaffold for the Management of Androgen Sensitive Prostatic Disorders#

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1. EXPERIMENTAL

1.1. Chemistry

General information

All solvents and reagents were commercial available and used without further purification. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates 60 GF$_{254}$ (Aldrich) to monitor the progress of the reaction. Electrospray ionization mass spectra (ESI-MS) were recorded on Ion Ttrap LCQ Advantage Max-IT (Thermo Electron Corporation). High-resolution mass spectra (HRMS) were recorded on a 6520 Agilent Q Tof LC MS/MS (accurate mass). $^1$H and $^{13}$C NMR spectra were done on Bruker Supercon Magnet Avance spectrometers at 300/400 and 75.4/100 MHz respectively in deuterated solvents taking TMS as internal reference (chemical shifts $\delta$ in ppm, $J$ in Hz.). IR spectra ($\nu_{\text{max}}$ in cm$^{-1}$) were taken on Perkin Elmer’s FT-IR RX1 PC spectrophotometer. Melting points were determined in open capillary tubes on the melting point apparatus. Elemental analyses were done on Carlo Erba EA-1108 micro analyzer/Vario ELIII C H N S analyzer. All compounds were analyzed for C, H, N and the results were within ±0.3% of calculated values. All compounds were characterized by TLC, $^1$H and $^{13}$C NMR, MS, and HRMS. Elemental analyses data meet the criteria of >95% purity.

2-Chloro-1-(4-(4-nitrophenyl)piperazin-1-yl)ethanone (8a)

The mixture of 1-(4-nitrophenyl)piperazine (7a, 1 g, 4.83 mmol), Et$_3$N (1.34 mL, 9.66 mmol) in dry DCM (15 mL) was stirred at 0 to 5 °C for 15 min under dry condition using CaCl$_2$ guard tube and then chloroacetyl chloride (0.76 mL, 9.66 mmol) in dry DCM (5 mL), was added dropwise within 1 h duration. Reaction mixture was further stirred at room temperature for 2 h. The resultant reaction mixture was concentrated under reduced pressure, dissolved in EtOAc (15 mL) and washed with distilled water (10 mL $\times$ 3) and organic layer was separated. The combined organic layer dried (anhyd. Na$_2$SO$_4$) and concentrated under reduced pressure. The obtained solid residue was recrystallized using EtOAc/Hexane to give the title compound as yellow solid (yield 92%); mp: 100-101 °C; IR (KBr) $\nu$ (cm$^{-1}$): 3012, 2853, 1653, 1596, 1500, 1442, 1389, 1327; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.14-8.19 (2H, m), 6.84-6.82 (2H, m), 4.11 (2H, s), 3.81-3.79 (2H, m), 3.74-3.72 (2H, m), 3.53-3.50 (2H, m), 3.47-3.45 (2H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.3, 154.3, 139.3, 125.9, 113.2, 47.1, 46.7, 45.4, 41.5, 40.6; HRMS (ESI positive)
m/z calcd. for C_{12}H_{14}ClN_{3}O_{3} [M+H]^+: 284.0802, found: 284.0799; Anal calcd. for C_{12}H_{14}ClN_{3}O_{3}: C, 50.80; H, 4.97; N, 14.81, found: C, 50.66; H, 4.73; N, 14.61.

Further the compounds 8b-e was synthesized using the procedure similar to compound 8a from the corresponding substituted piperazines.

2-Chloro-1-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (8b)
The title compound was synthesized from 2-(piperazine-1-yl)pyrimidine (7b) as off-white solid (yield 81.5%); mp: 75-76 °C; IR (KBr) ν (cm\(^{-1}\)): 3018, 2399, 1647, 1586, 1552, 1497, 1442, 1358; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 8.35 (2H, d, \(J = 4.4\) Hz), 6.58 (1H, t, \(J = 4.4\) Hz), 4.14-4.12 (2H, m), 3.92-3.85 (4H, m), 3.73-3.71 (2H, m), 3.62-3.61 (2H, m); HRMS (ESI positive) m/z calcd. for C\(_{10}\)H\(_{13}\)ClN\(_{4}\)O [M+H]^+: 241.0856, found: 241.0855; Anal calcd. for C\(_{10}\)H\(_{13}\)ClN\(_{4}\)O: C, 49.90; H, 5.44; N, 23.28, found: C, 49.66; H, 5.28; N, 23.02.

2-Chloro-1-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone (8c)
The title compound was synthesized from 1-(2-methoxyphenyl)piperazine (7c) as off-white solid (yield 54%); mp: 78-79 °C; IR (KBr) ν (cm\(^{-1}\)): 3018, 2832, 2401, 1648, 1595, 1500, 1450; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.07-7.02 (1H, m), 6.96-6.87 (3H, m), 4.11 (2H, bs), 3.88 (3H, s), 3.81 (2H, t, \(J = 4.2\) Hz), 3.71-3.68 (2H, m) 3.12-3.03 (4H, m); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 165.1, 152.2, 140.3, 123.7, 121.0, 118.5, 111.4, 55.4, 50.8, 50.3, 46.5, 42.3, 40.9; HRMS (ESI positive) m/z calcd. for C\(_{13}\)H\(_{17}\)ClN\(_{2}\)O\(_2\) [M+H]^+: 269.1057, found: 269.1052; Anal calcd. for C\(_{13}\)H\(_{17}\)ClN\(_{2}\)O\(_2\): C, 58.10; H, 6.38; N, 10.42, found: C, 58.29; H, 6.58; N, 10.69.

2-Chloro-1-(4-(4-nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (8d)
The title compound was synthesized from 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (7d) to give pure yellow oily compound (yield 80%); IR (neat): ν (cm\(^{-1}\)): 3019, 2925, 1648, 1613, 1526, 1425, 1384, 1347; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.51 (1H, d, \(J = 2.6\) Hz), 8.36 (1H, d, \(J = 2.6\) Hz), 7.34 (1H, d, \(J = 8.9\) Hz), 4.10 (2H, bs), 3.79 (2H, t, \(J = 4.8\) Hz ), 3.71-3.69 (2H, m), 3.14 (2H, t, \(J = 4.7\) Hz), 3.09 (2H, t, \(J = 4.8\) Hz); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 165.2, 156.5, 143.3, 128.0, 126.0, 125.7, 124.2, 123.4, 52.9, 52.3, 46.3, 42.1, 40.8; HRMS (ESI positive) m/z calcd. for C\(_{13}\)H\(_{13}\)ClF\(_3\)N\(_3\)O\(_3\) [M+H]^+: 352.0676, found: 352.0675; Anal calcd. for C\(_{13}\)H\(_{13}\)ClF\(_3\)N\(_3\)O\(_3\): C, 44.39; H, 3.73; N, 11.95, found: C, 44.56; H, 3.91; N, 12.19.

2-Chloro-1-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (8e)
The title compound was prepared from 1-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (7e) as pure yellow oily compound (yield 89%); IR (neat) ν (cm\(^{-1}\)): 3429, 3016, 2967, 1654, 1535, 1436,
1H NMR (300 MHz, CDCl₃):  δ 8.11 (1H, bs), 7.73 (1H, d, J = 8.8 Hz, Ar-H), 7.21 (1H, d, J = 8.7 Hz), 4.11 (2H, bs), 3.83 (2H, bs), 3.73 (2H, bs), 3.21-3.20 (4H, m); HRMS (ESI positive) m/z calcd for C₁₃H₁₃ClF₃N₃O₃ [M+H]⁺: 352.0676, found: 352.0657; Anal calcd for C₁₃H₁₃ClF₃N₃O₃: C, 44.39; H, 3.73; N, 11.95, found: C, 44.15; H, 3.51; N, 11.70.

1, 2-Bis(4-(4-nitrophenyl)piperazin-1-yl)ethanone (9a)

To the mixture of 8a (0.3 g, 1.06 mmol) and Et₃N (0.3 mL, 2.12 mmol) in CHCl₃ (5 mL) was added 1-(4-nitrophenyl)piperazine (7a, 0.320 g, 1.59 mmol) in 5 mL CHCl₃ dropwise within 1 h. After complete addition reaction mixture was further stirred in an oil bath at 80-85 °C for 15 h. The reaction mixture was cooled, washed with water (5 mL × 3) and the organic layer was separated. Combined organic layer was dried (anhyd. Na₂SO₄) and concentrated under reduced pressure in rotavapor. The solid obtained was purified by recrystallization using EtOAc/Hexane which furnished yellow crystals (yield 81%); mp: 156-157 °C; IR (KBr) ν (cm⁻¹): 3019, 2399, 1640, 1597, 1506, 1423, 1330; ¹H NMR (400 MHz, CDCl₃):  δ 8.14-8.09 (4H, m), 6.84-6.81 (4H, m), 3.84-3.83 (4H, m), 3.49-3.44 (8H, m), 3.33 (2H, s), 2.72 (4H, t, J = 5.0 Hz); ¹³C NMR (75.4 MHz, CDCl₃):  δ 167.7, 154.7, 154.3, 138.8, 138.4, 125.9, 125.8, 112.9, 112.7, 60.8, 52.5, 46.9, 46.7, 44.6; HRMS (ESI positive) m/z calcd. for C₂₂H₂₆N₆O₅ [M+H]⁺: 455.2043, found: 455.2034; Anal calcd. for C₂₂H₂₆N₆O₅: C, 58.14; H, 5.77; N, 18.49, found: C, 58.31; H, 5.92; N, 18.66.

The compounds 9b-n were prepared by adopting the procedure similar to 9a using their respective precursors.

2-(4-(2-Methoxyphenyl)piperazin-1-yl)-1-(4-(4-nitrophenyl)piperazin-1-yl)ethanone (9b)

The title compound was synthesized from 8a and 1-(2-methoxyphenyl)piperazine (7c) as yellow solid (yield 78%); mp: 123-124 °C; IR (KBr) ν (cm⁻¹): 3020, 2833, 2401, 1641, 1596, 1503, 1445, 132; ¹H NMR (300 MHz, CDCl₃):  δ 8.14 (2H, d, J = 8.5 Hz), 7.01-6.98 (1H, m), 6.93 (2H, bs), 6.88-6.82 (3H, m), 3.86-3.80 (7H, m), 3.48-3.44 (4H, m), 3.31 (2H, s), 3.10 (4H, bs), 2.73 (4H, bs); ¹³C NMR (100 MHz, CDCl₃):  δ 168.3, 154.5, 152.2, 141.0, 138.9, 125.9, 123.0, 120.9, 118.1, 112.9, 111.3, 61.6, 55.4, 53.4, 50.6, 47.4, 46.8, 44.8, 41.2; HRMS (ESI positive) m/z calcd. for C₂₃H₂₉N₅O₄ [M+H]⁺: 440.2298, found: 440.2283; Anal calcd. for C₂₃H₂₉N₅O₄: C, 62.85; H, 6.65; N, 15.93, found: C, 62.63; H, 6.20; N, 15.82.

1-(4-(4-Nitrophenyl)piperazin-1-yl)-2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9c)
The title compound was synthesized from 8a and 2-(piperazine-1-yl)pyrimidine (7b) as yellow solid (yield 68%); mp: 151-152 °C; IR (KBr) $\nu$ (cm$^{-1}$): 3020, 2401, 1642, 1592, 1506, 1443, 1329; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.31 (2H, d, $J = 4.5$ Hz), 8.13 (2H, d, $J = 8.6$ Hz), 6.84 (2H, d, $J = 8.7$ Hz), 6.51 (1H, t, $J = 4.5$ Hz), 3.85 (8H, bs), 3.49-3.46 (4H, m), 3.29 (2H, s), 2.60 (4H, bs); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 168.0, 161.6, 157.6, 154.4, 138.9, 125.9, 112.9, 110.0, 61.4, 53.0, 47.3, 46.8, 44.8, 43.6, 41.2; HRMS (ESI positive) m/z calcd. for C$_{20}$H$_{25}$N$_7$O$_3$ [M+H]$^+$: 412.2097, found: 412.2096; Anal calcd. for C$_{20}$H$_{25}$N$_7$O$_3$: C, 58.38; H, 6.12; N, 23.83, found: C, 58.60; H, 6.31; N, 23.99.

1-(4-(4-Nitrophenyl)piperazin-1-yl)-2-(4-(pyridin-2-yl)piperazin-1-yl)ethanone (9d)

The title compound was synthesized from 8a and 1-(pyridine-2-yl)piperazine (10a) as yellow solid (yield 65%); mp: 135-136 °C; IR (KBr) $\nu$ (cm$^{-1}$): 3018, 2402, 1699, 1641, 1596, 1485, 1439, 1384, 1325; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.19-8.13 (3H, m), 7.49 (1H, t, $J = 7.4$ Hz), 6.83 (2H, d, $J = 8.8$ Hz), 6.67-6.62 (2H, m), 3.84 (4H, d, $J = 16.2$ Hz), 3.56-3.45 (8H, m), 3.30 (2H, bs), 2.66-2.65 (4H, m); $^{13}$C NMR (75.4 MHz, CDCl$_3$): $\delta$ 168.0, 161.6, 157.6, 154.4, 138.9, 125.9, 110.0, 61.4, 53.0, 47.3, 46.8, 44.8, 43.6, 41.2; HRMS (ESI positive) m/z calcd. for C$_{21}$H$_{26}$N$_6$O$_3$ [M+H]$^+$: 411.2145, found: 411.2160; Anal calcd. for C$_{21}$H$_{26}$N$_6$O$_3$: C, 61.45; H, 6.38; N, 20.47, found: C, 61.62; H, 6.55; N, 20.69.

2-(4-(4-Nitrophenyl)piperazin-1-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9e)

The title compound was synthesized from 8b and 1-(4-nitrophenyl)piperazine (7a) as yellow solid (yield 65%); mp: 174-175 °C; IR (KBr) $\nu$ (cm$^{-1}$): 3020, 2401, 1642, 1592, 1506, 1443, 1329; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.33 (2H, d, $J = 4.6$ Hz), 8.11 (2H, d, $J = 9.1$ Hz), 6.82 (2H, d, $J = 8.6$ Hz), 6.57-6.54 (1H, m), 3.85 (4H, bs), 3.70 (4H, bs), 3.45 (4H, bs), 3.32 (2H, s), 2.72-2.71 (4H, m); ESI-MS m/z calcd for C$_{20}$H$_{25}$N$_7$O$_3$ [M+H]$^+$: 412; Anal calcd. for C$_{20}$H$_{25}$N$_7$O$_3$: C, 58.38; H, 6.12; N, 23.83, found: C, 58.63; H, 6.28; N, 23.99.

1, 2-Bis(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9f)

The title compound was synthesized from 8b and 2-(piperazine-1-yl)pyrimidine (7b) as off-white solid (yield 63%); mp: 150-151 °C; IR (KBr) $\nu$ (cm$^{-1}$): 3020, 2401, 1639, 1592, 1502, 1441, 1329; $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 8.33 (2H, d, $J = 4.6$ Hz), 8.11 (2H, d, $J = 9.1$ Hz), 6.82 (2H, d, $J = 8.6$ Hz), 6.57-6.54 (1H, m), 3.85 (4H, bs), 3.70 (4H, bs), 3.45 (4H, bs), 3.32 (2H, s), 2.72-2.71 (4H, m); ESI-MS m/z calcd for C$_{20}$H$_{25}$N$_7$O$_3$ [M+H]$^+$: 412; Anal calcd. for C$_{20}$H$_{25}$N$_7$O$_3$: C, 58.38; H, 6.12; N, 23.83, found: C, 58.63; H, 6.28; N, 23.99.
m/z calcd. for C\textsubscript{18}H\textsubscript{24}N\textsubscript{8}O [M+H]\textsuperscript{+}: 369.2151, found: 369.2142; Anal calcd. for C\textsubscript{18}H\textsubscript{24}N\textsubscript{8}O: C, 58.68; H, 6.57; N, 30.41, found C, 58.86; H, 6.66; N, 30.58.

2-(4-(2-Methoxyphenyl)piperazin-1-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9g)
The title compound was synthesized from 8b and 1-(2-methoxyphenyl)piperazine (7c) as off-white solid (yield 78%); mp: 96-97 °C; IR (KBr) ν (cm\textsuperscript{-1}): 3430, 3017, 1637, 1586, 1551, 1498, 1447, 1305; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 8.32 (2H, d, J = 4.5 Hz), 6.99-6.84 (4H, m), 6.53 (1H, t, J = 4.6 Hz), 3.85 (7H, bs), 3.72 (4H, bs) 3.30 (2H, s), 3.10 (4H, bs), 2.73 (4H, bs); \textsuperscript{13}C NMR (75.4 MHz, CDCl\textsubscript{3}): δ 168.4, 161.6, 157.8, 152.3, 141.2, 123.0, 121.0, 118.3, 111.4, 110.5, 61.6, 55.4, 53.4, 50.6, 45.6, 44.2, 43.7, 41.8; HRMS (ESI positive) m/z calcd. for C\textsubscript{21}H\textsubscript{28}N\textsubscript{6}O\textsubscript{2} [M+H]\textsuperscript{+}: 397.2352, found: 397.2351; Anal calcd. for C\textsubscript{21}H\textsubscript{28}N\textsubscript{6}O\textsubscript{2}: C, 63.62; H, 7.12; N, 21.20, found: C, 63.76; H, 7.22; N, 21.38.

2-(4-(Pyridine-2-yl)piperazin-1-yl)-1-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9h)
The title compound was synthesized from 8b and 1-(pyridine-2-yl)piperazine (10a) as white solid (yield 80%); mp: 123-124 °C; IR (KBr) ν (cm\textsuperscript{-1}): 3020, 2401, 1593, 1519, 1478, 1431; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 8.32 (2H, d, J = 4.6 Hz), 8.18 (1H, d, J = 4.1 Hz), 7.50-7.45 (1H, m), 6.66-6.61 (2H, m), 6.55-6.52 (1H, m), 3.85-3.84 (4H, m), 3.73-3.71 (4H, m), 3.57-3.56 (4H, m), 3.29 (2H, s), 2.67-2.65 (4H, m); \textsuperscript{13}C NMR (75.4 MHz, CDCl\textsubscript{3}): δ 168.2, 161.7, 159.6, 157.9, 148.1, 137.6, 113.6, 110.6, 107.3, 61.7, 53.1, 45.7, 45.4, 44.3, 43.8, 41.9; HRMS (ESI positive) m/z calcd. for C\textsubscript{19}H\textsubscript{25}N\textsubscript{7}O [M+H]\textsuperscript{+}: 368.2199, found: 368.2185; Anal calcd. for C\textsubscript{19}H\textsubscript{25}N\textsubscript{7}O: C, 62.10; H, 6.86; N, 26.68, found: C, 62.28; H, 6.95; N, 26.86.

1-(4-(2-Methoxyphenyl)piperazin-1-yl)-2-(4-(4-nitrophenyl)piperazin-1-yl)ethanone (9i)
The title compound was synthesized from 8c and 1-(4-nitrophenyl)piperazine (7a) as yellow solid (yield 84%); mp: 85-86 °C; IR (KBr) ν (cm\textsuperscript{-1}): 3018, 1633, 1597, 1500, 1329; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): δ 8.13 (2H, d, J = 8.1 Hz), 7.06-7.03 (1H, m), 6.94-6.89 (3H, m), 6.83 (2H, d, J = 8.6 Hz), 3.89 (3H, s), 3.82-3.80 (4H, m), 3.46 (4H, bs), 3.32 (2H, s), 3.08-3.05 (4H, m), 2.72 (4H, bs); \textsuperscript{13}C NMR (75.4 MHz, CDCl\textsubscript{3}): 167.4, 154.8, 152.2, 140.5, 138.5, 125.9, 123.6, 121.0, 118.3, 112.7, 111.3, 60.8, 55.4, 52.6, 51.2, 50.7, 47.0; HRMS (ESI positive) m/z calcd. for C\textsubscript{23}H\textsubscript{29}N\textsubscript{5}O\textsubscript{4} [M+H]\textsuperscript{+}: 440.2298, found: 440.2274; Anal calcd. for C\textsubscript{23}H\textsubscript{29}N\textsubscript{5}O\textsubscript{4}: C, 62.85; H, 6.65; N, 15.93, found: C, 62.99; H, 6.76; N, 16.09.

1, 2-Bis(4-(2-methoxyphenyl)piperazin-1-yl)ethanone (9j)
The title compound was synthesized from 8c and 1-(2-methoxyphenyl)piperazine (7c) as brown crystals (yield 63%); mp: 144-145 °C; IR (KBr) ν (cm⁻¹): 3019, 2854, 1632, 1500, 1465, 1384; ¹H NMR (400 MHz, CDCl₃): δ 7.03-6.97 (2H, m), 6.94-6.84 (6H, m), 3.88-3.80 (10H, m), 3.30 (2H, s), 3.09-3.01 (8H, m) 2.73 (4H, bs); ¹³C NMR (75.4 MHz, CDCl₃): δ 167.9, 152.2, 141.2, 140.7, 123.5, 122.9, 121.0, 120.9, 118.4, 118.2, 111.4, 111.3, 61.4, 55.5, 55.4, 53.3, 51.3, 50.7, 50.5, 45.9, 42.0; HRMS (ESI positive) m/z calcd. for C₂₄H₃₂N₄O₃[M+H]^+: 425.2553, found: 425.2551; Anal calcd. for C₂₄H₃₂N₄O₃: C, 67.90; H, 7.60; N, 13.20, found: C, 67.79; H, 7.48; N, 13.03.

1-(4-(2-Methoxyphenyl)piperazin-1-yl)-2-(4-(pyridin-2-yl)piperazin-1-yl)ethanone (9k)
The title compound was synthesized from 8c and 1-(pyridine-2-yl)piperazine (10a) as brown crystals (yield 55%); mp: 124-125 °C; IR (KBr) ν (cm⁻¹): 3018, 2833, 2402, 1634, 1478, 1439, 1385; ¹H NMR (400 MHz, CDCl₃): δ 8.18-8.16 (1H, m), 7.49-7.44 (1H, m), 7.04-7.00 (1H, m), 6.94-6.86 (3H, m), 6.65-6.60 (2H, m) 3.87 (3H, s), 3.83-3.79 (4H, m), 3.55 (4H, t, J = 4.5 Hz), 3.28 (2H, s), 3.06 (2H, t, J = 4.8 Hz), 3.02 (2H, t, J = 5.0 Hz), 2.65 (4H, t, J = 5.0 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 167.8, 159.4, 152.2, 147.8, 140.7, 137.4, 123.5, 121.0, 118.4, 113.4, 111.3, 107.1, 61.3, 55.4, 52.9, 51.2, 50.7, 45.9, 45.2, 42.0; HRMS (ESI positive) m/z calcd. for C₂₂H₂₉N₅O₂[M+H]^+: 396.2400, found: 396.2387; Anal calcd. for C₂₂H₂₉N₅O₂: C, 66.81; H, 7.39; N, 17.71, found: C, 66.99; H, 7.52; N, 17.96.

1-(4-(2-Methoxyphenyl)piperazin-1-yl)-2-(4-(pyrimidin-2-yl)piperazin-1-yl)ethanone (9l)
The title compound was synthesized from 8c and 2-(piperazine-1-yl)pyrimidine (7b) as brown crystals (yield 67%); mp: 129-130 °C; IR (KBr) ν (cm⁻¹): 3022, 1634, 1587, 1549, 1500, 1447, 1384, 1307; ¹H NMR (400 MHz, CDCl₃): δ 8.30 (2H, d, J = 4.7 Hz), 7.05-7.01 (1H, m), 6.95-6.87 (3H, m), 6.50-6.47 (1H, m), 3.88 (3H, s), 3.86-3.82 (8H, m), 3.28 (2H, s), 3.09-3.02 (4H, m), 2.60 (4H, t, J = 4.9 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 167.8, 161.6, 157.6, 152.2, 140.7, 123.5, 121.0, 118.4, 111.3, 109.9, 61.3, 55.4, 52.9, 51.2, 50.7, 46.0, 43.6, 42.0; HRMS (ESI positive) m/z calcd. for C₂₁H₂₉N₆O₂[M+H]^+: 397.2352, found: 397.2351; Anal calcd. for C₂₁H₂₉N₆O₂: C, 66.81; H, 7.39; N, 17.71, found: C, 66.99; H, 7.52; N, 17.96.

1, 2-Bis(4-(4-nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (9m)
The title compound was synthesized from 8d and 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (7d) as yellow oily compound (yield 86%); IR (neat) ν (cm⁻¹): 3022, 1634, 1587, 1549, 1500, 1447, 1384, 1307; ¹H NMR (400 MHz, CDCl₃): δ 8.30 (2H, d, J = 4.7 Hz), 7.05-7.01 (1H, m), 6.95-6.87 (3H, m), 6.50-6.47 (1H, m), 3.88 (3H, s), 3.86-3.82 (8H, m), 3.28 (2H, s), 3.09-3.02 (4H, m), 2.60 (4H, t, J = 4.9 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 167.8, 161.6, 157.6, 152.2, 140.7, 123.5, 121.0, 118.4, 111.3, 109.9, 61.3, 55.4, 52.9, 51.2, 50.7, 46.0, 43.6, 42.0; HRMS (ESI positive) m/z calcd. for C₂₁H₂₉N₆O₂[M+H]^+: 397.2352, found: 397.2351; Anal calcd. for C₂₁H₂₉N₆O₂: C, 63.62; H, 7.12; N, 21.20, found: C, 63.48; H, 7.00; N, 21.06.

1, 2-Bis(4-(4-nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (9m)
The title compound was synthesized from 8d and 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (7d) as yellow oily compound (yield 86%); IR (neat) ν (cm⁻¹):
1H NMR (400 MHz, CDCl$_3$): $\delta$ 8.56-8.51 (2H, m), 8.39-8.32 (2H, m), 7.34-7.30 (2H, m), 3.82 (4H, bs), 3.34 (2H, s), 3.21-3.19 (4H, m), 3.13-3.10 (4H, m), 2.76-2.75 (4H, m); $^{13}$C NMR (75.4 MHz, CDCl$_3$): $\delta$ 167.8, 156.8, 156.5, 143.0, 142.2, 128.0, 127.8, 124.6, 124.5, 124.4, 124.3, 123.0, 122.3, 60.8, 53.0, 52.6, 45.6, 41.7;

HRMS (ESI positive) m/z calcd. for C$_{24}$H$_{24}$F$_6$N$_6$O$_5$ [M+H]$^+$: 591.1791, found: 591.1790; Anal calcd. for C$_{24}$H$_{24}$F$_6$N$_6$O$_5$: C, 48.82; H, 4.10; N, 14.23, found: C, 48.66; H, 4.05; N, 14.06.

1, 2-Bis(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)ethanone (9n)

The title compound was synthesized from 8e and 1-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (7e) as pure yellow oily compound (yield 83%); IR (neat) v (cm$^{-1}$): 3020, 2928, 2401, 1672, 1532, 1428, 1326; 1H NMR (500 MHz, CDCl$_3$) $\delta$ 8.03-7.99 (2H, m), 7.63-7.62 (2H, m), 7.20-7.13 (2H, m), 3.71-3.57 (6H, m), 3.42-3.33 (4H, m) 3.11 (8H, bs);

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 164.9, 147.6, 147.4, 141.5, 141.2, 130.4, 124.1, 121.6, 121.1, 58.5, 52.7, 51.3, 50.7, 49.6, 49.4, 45.0, 41.6; HRMS (ESI positive) m/z calcd. for C$_{24}$H$_{24}$F$_6$N$_6$O$_5$ [M+H]$^+$: 591.1791, found: 591.1776; Anal calcd. for C$_{24}$H$_{24}$F$_6$N$_6$O$_5$: C, 48.82; H, 4.10; N, 14.23, found: C, 48.96; H, 4.25; N, 14.36.

4-Nitrophenyl 4-(pyridin-2-yl)piperazine-1-carboxylate (11a)

To the mixture of 1-(pyridin-2-yl)piperazine (10a, 0.4 mL, 2.74 mmol) and Et$_3$N (0.6 mL, 4.11 mmol) in DCM (10 mL) was added 4-nitrophenyl chloroformate (718 mg, 3.56 mmol) in 5 mL DCM) dropwise in 0.5 h duration in ice-bath (0-5 °C). After complete addition reaction mixture was further stirred at room temperature for 1 h. It was concentrated under reduced pressure and extracted with EtOAc (10 mL x 3). EtOAc layer was washed with water (5 mL x 3), dried (anhyd. Na$_2$SO$_4$) and concentrated on rotavapor. The solid obtained was further purified by recrystallization using EtOAc/Hexane as yellow solid (yield 87%); mp: 138-139 °C; IR (KBr) v (cm$^{-1}$): 3021, 2483, 1723, 1657, 1594, 1340; 1H NMR (400 MHz, CDCl$_3$) $\delta$ 8.23-8.20 (3H, m), 7.56-7.52 (1H, m), 7.34-7.30 (2H, m), 6.72-6.69 (2H, m), 3.71-3.63 (8H, m); $^{13}$C NMR (75.4 MHz, CDCl$_3$): $\delta$ 163.5, 158.9, 156.0, 152.3, 147.8, 144.8, 137.9, 125.0, 122.3, 115.5, 114.1, 107.6, 58.0,45.0, 44.3; ESI-MS m/z calcd for C$_{16}$H$_{16}$N$_4$O$_4$ [M+H]$^+$: 329; Anal calcd for C$_{16}$H$_{16}$N$_4$O$_4$: C, 58.53; H, 4.91; N, 17.06; Found C, 58.61; H, 4.85; N, 17.18.

4-Nitrophenyl 4-(4-nitrophenyl)piperazine-1-carboxylate (11b)

The title compound was synthesized using the procedure similar to 11a from 1-(4-nitrophenyl)piperazine (7a) as yellow solid (yield 89%); mp: 125-126 °C; IR (neat) v (cm$^{-1}$):
3020, 2836, 1611, 1434, 1346; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.18 (2H, d, \(J = 8.2\) Hz), 8.07 (2H, d, \(J = 8.6\) Hz), 6.78 (2H, d, \(J = 8.5\) Hz), 3.76 (4H, d, \(J = 41.3\) Hz), 3.48 (4H, bs); \(^{13}\)C NMR (75.4 MHz, CDCl\(_3\)): \(\delta\) 162.2, 155.8, 154.4, 152.3, 145.0, 139.0, 126.1, 126.0, 125.1, 115.6, 113.1, 46.7, 43.9, 43.3; ESI-MS \(m/z\) calcd for C\(_{17}\)H\(_{16}\)N\(_4\)O\(_6\) [M+H\(^+\)]: 373; Anal calcd. for C\(_{17}\)H\(_{16}\)N\(_4\)O\(_6\): C, 54.84; H, 4.83; N, 15.05, found: C, 54.99; H, 4.66; N, 15.26.

(4-(4-Nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (12a)
The mixture of compound 11a (411 mg, 1.25 mmol) and 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (7d, 0.23 mL, 1.62 mmol) in DMF (10 mL) was stirred at 100-110 °C for 60 h. The reaction mixture was cooled and water (25 mL) was added. The crude product was extracted with EtOAc (10 mL x 3). EtOAc layer was washed with water (5 mL x 3), dried (anhyd. Na\(_2\)SO\(_4\)) and concentrated under reduced pressure. The product obtained was purified over column chromatography (230-400 mesh) using EtOAc/Hexane as eluent as yellow solid (yield 65%); mp: 162-163 °C; IR (neat) \(\nu\) (cm\(^{-1}\)): 2924, 2854, 1596, 1482, 1426, 1338; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.54 (1H, d, \(J = 2.7\) Hz), 8.37 (1H, dd, \(J = 2.6, 8.9\) Hz), 8.21 (1H, dd, \(J = 1.9, 5.3\) Hz), 7.52-7.50 (1H, m), 7.34-7.32 (1H, m), 6.69-6.66 (2H, m), 3.60-3.57 (4H, m), 3.52 (4H, t, \(J = 4.6\) Hz), 3.46-3.43 (4H, m), 3.13 (4H, t, \(J = 4.7\) Hz); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 163.6, 159.3, 156.8, 147.9, 142.8, 137.6, 127.9, 125.5, 125.2, 124.5, 124.4, 124.3, 122.9, 113.8, 107.2, 52.6, 46.7, 46.5, 45.1; HRMS (ESI positive) \(m/z\) calcd. for C\(_{21}\)H\(_{23}\)F\(_3\)N\(_6\)O\(_3\) [M+H\(^+\)]: 465.1862, found: 465.1859; Anal calcd. for C\(_{21}\)H\(_{23}\)F\(_3\)N\(_6\)O\(_3\): C, 54.31; H, 4.99; N, 18.09, found: C, 54.52; H, 4.89; N, 18.16.
The following compounds (12b-g) were prepared using a procedure similar to that described for compound 12a from corresponding arylpiperazine and compound 11a-b.

(4-(4-Nitro-3-(trifluoromethyl)phenyl)piperazin-1-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (12b)
The title compound was synthesized from 11a and 1-(4-nitro-3-(trifluoromethyl)phenyl)piperazine as yellow solid (yield 76%); mp: 133-134 °C; IR (neat) \(\nu\) (cm\(^{-1}\)): 3020, 2927, 2401, 1598, 1522, 1478, 1427, 1338; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.21-8.19 (1H, m), 8.02 (1H, d, \(J = 9.2\) Hz), 7.54-7.50 (1H, m), 7.16 (1H, d, \(J = 2.4\) Hz), 6.95 (1H, dd, \(J = 2.7, 9.2\) Hz), 6.69-6.66 (2H, m), 3.60-3.45 (16H, m); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 163.5,
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159.2, 153.1, 147.9, 137.6, 128.5, 126.3, 126.0, 115.0, 113.9, 112.3, 112.2, 107.3, 46.7, 46.4, 46.1, 45.0; HRMS (ESI positive) m/z calcd. for C$_{21}$H$_{23}$F$_3$N$_6$O$_3$ [M+H]$^+$: 465.1862, found: 465.1858; Anal calcd. for C$_{21}$H$_{23}$F$_3$N$_6$O$_3$: C, 54.31; H, 4.99; N, 18.09, found: C, 54.42; H, 4.81; N, 18.20.

**Bis(4-(pyridin-2-yl)piperazin-1-yl)methanone (12c)**

The title compound was synthesized from 11a and 1-(pyridin-2-yl)piperazine (10a) as pure yellow solid compound (yield 73%); mp: 142-143 °C; IR (neat) ν (cm$^{-1}$): 3010, 2925, 2854, 2462, 1664, 1597, 1314; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19-8.11 (2H, m), 7.51-7.47 (2H, m), 6.66-6.63 (4H, m), 3.65-3.41 (16H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.8, 160.8, 159.3, 147.9, 137.5, 113.7, 107.2, 46.5, 45.9, 45.2, 45.0; HRMS (ESI positive) m/z calcd. for C$_{19}$H$_{24}$N$_6$O [M+H]$^+$: 353.2090, found: 353.2090; Anal calcd. for C$_{19}$H$_{24}$N$_6$O: C, 64.75; H, 6.86; N, 23.85, found: C, 64.60; H, 6.77; N, 23.94.

**Bis(4-(4-nitrophenyl)piperazin-1-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (12d)**

The title compound was synthesized from 11a and 1-(4-nitrophenyl)piperazine (7a) as solid compound of yellow colour (yield 72%); mp: 130-131 °C; IR (neat) ν (cm$^{-1}$): 3018, 2926, 2402, 1673, 1522, 1437; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.21-8.19 (1H, m), 8.15-8.12 (2H, m), 7.54-7.49 (1H, m), 6.85-6.82 (2H, m), 3.60-3.57 (4H, m), 3.50-3.44 (12H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.6, 159.2, 154.6, 147.9, 138.8, 137.6, 125.9, 113.8, 112.8, 107.3, 46.7, 46.4, 46.2, 45.0; HRMS (ESI positive) m/z calcd. for C$_{20}$H$_{24}$N$_6$O$_3$ [M+H]$^+$: 397.1988, found: 397.1985; Anal calcd. for C$_{20}$H$_{24}$N$_6$O$_3$: C, 60.59; H, 6.10; N, 21.20, found: C, 60.44; H, 6.29; N, 21.36.

**Bis(4-(4-nitrophenyl)piperazin-1-yl)methanone (12e)**

The title compound was synthesized from 11b and 1-(4-nitrophenyl)piperazine (7a) as yellow solid (yield 76%); mp: 145-146 °C; IR (neat) ν (cm$^{-1}$): 3020, 2926, 2401, 1638, 1596, 1505; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.15-8.12 (4H, m), 6.86-6.82 (4H, m), 3.53-3.48 (16H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.3, 154.6, 139.0, 125.9, 112.9, 112.8, 107.3, 46.7, 46.4, 46.2, 45.0; HRMS (ESI positive) m/z calcd. for C$_{21}$H$_{24}$N$_6$O$_5$ [M+H]$^+$: 441.1886, found: 441.1876; Anal calcd. for C$_{21}$H$_{24}$N$_6$O$_5$: C, 57.26; H, 5.49; N, 19.08, found: C, 57.44; H, 5.31; N, 19.19.

**(4-(4-Nitro-2-(trifluoromethyl)phenyl)piperazin-1-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (12f)**

The title compound was synthesized from 11b and 1-(4-nitrophenyl)piperazine (7a) as yellow solid (yield 76%); mp: 145-146 °C; IR (neat) ν (cm$^{-1}$): 3020, 2926, 2401, 1638, 1596, 1505; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.15-8.12 (4H, m), 6.86-6.82 (4H, m), 3.53-3.48 (16H, m); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 163.3, 154.6, 139.0, 125.9, 112.9, 46.8, 46.1; HRMS (ESI positive) m/z calcd. for C$_{21}$H$_{24}$N$_6$O$_5$ [M+H]$^+$: 441.1886, found: 441.1876; Anal calcd. for C$_{21}$H$_{24}$N$_6$O$_5$: C, 57.26; H, 5.49; N, 19.08, found: C, 57.44; H, 5.31; N, 19.19.
The title compound was synthesized from 11b and 1-(4-nitro-2-(trifluoromethyl)phenyl)piperazine (7d) as yellow solid compound (yield 70%); mp: 137-138 °C; IR (neat) ν (cm⁻¹): 3021, 1596, 1407, 1328; ¹H NMR (400 MHz, CDCl₃): δ 8.53 (1H, d, J = 2.6 Hz), 8.36 (1H, dd, J = 2.6, 8.9 Hz), 8.14-8.11 (2H, m), 7.33 (1H, m, J = 8.9 Hz), 6.85-6.83 (2H, m), 3.55-3.47 (12H, m), 3.14 (4H, t, J = 4.8 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 163.3, 156.7, 154.6, 142.9, 138.9, 128.0, 125.9, 124.5, 124.4, 124.3, 123.0, 112.9, 52.6, 46.8, 46.6, 46.2; HRMS (ESI positive) m/z calcd. for C₂₂H₂₃F₃N₆O₅[M+H]⁺: 509.1760, found: 509.1739; Anal calcd. for C₂₂H₂₃F₃N₆O₅: C, 51.97; H, 4.56; N, 16.53, found: C, 51.77; H, 4.42; N, 16.62.

(4-(2-Nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (12g)

The title compound was synthesized from 11b and 1-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (7e) as yellow solid compound (yield 74%); mp: 141-142 °C; IR (neat) ν (cm⁻¹): 3016, 2433, 1627, 1404, 1326; ¹H NMR (400 MHz, CDCl₃): δ 8.15-8.09 (3H, m), 7.72 (1H, dd, J = 1.9, 8.7 Hz), 7.20 (1H, d, J = 8.6 Hz), 6.84 (2H, d, J = 9.4 Hz), 3.55-3.48 (12H, m), 3.20 (4H, t, J = 5 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 163.2, 154.6, 147.9, 141.0, 138.9, 130.4, 130.3, 125.9, 124.2, 124.1, 123.3, 120.8, 112.9, 50.7, 46.8, 46.3, 46.2; HRMS (ESI positive) m/z calcd. for C₂₂H₂₃F₃N₆O₅[M+H]⁺: 509.1760, found: 509.1743; Anal calcd. for C₂₂H₂₃F₃N₆O₅: C, 51.97; H, 4.56; N, 16.53, found: C, 51.81; H, 4.62; N, 16.44.

1.2. BIOLOGY

1.2.1. Cell cultures

The prostate cancer PC3 and DU145, monkey kidney Cos-7, and LNCaP cells were procured from National Centre for Cell sciences (NCCS, Pune, India). Human prostate cancer cells, PC3 and DU145, were maintained in DMEM/ HAM’S F-12 medium (Sigma–Aldrich, St. Louis, MO), and Cos-7 cells were grown in DMEM medium (Sigma–Aldrich, St. Louis, MO) supplemented with 10% FBS and 0.01% antibiotic/antimycotic solutions. The LNCaP cells were grown in Roswell Park Memorial Institute (RPMI) supplemented with 12.5% fetal bovine serum (charcoal stripped, Life Technologies Inc), and 0.01% antibiotic/antimycotic solutions in an atmosphere of 5% CO₂/95% air at 37 °C.

Ready-to-Assay α₁a Adrenergic Receptor GPCR frozen cells were used for rapid calcium assays and procured from Millipore (cat no. HTS087RTA).

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1.2.2. Cell proliferation assay

The prostate cancer and normal cells were seeded in 96-well plates at a density of $2 \times 10^4$ cells/well and allowed 24 h for attachment. The treatment were proceeded in triplicate with flutamide (Stock 10 mM; Sigma-Aldrich, USA) and with compounds (Stock 10 mM) at concentrations ranging from 2.5 µM to 80 µM in 2% charcoal stripped serum for 24 h at 37 °C in 5% CO$_2$ atmosphere. The stock solution of flutamide (10 mM) was dissolved in molecular grade dimethyl sulfoxide (DMSO) and diluted with culture medium to different concentrations. Final concentration of DMSO was not more than 0.05%. Controls were treated with DMSO (0.05% in culture medium). After 24 h of incubation in CO$_2$ incubator, 5 µl of 5mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] was added to the cells. After incubation of 3 h, the formazan crystals formed in viable cells were dissolved in DMSO with gentle shaking on a plate shaker for 5 min. The absorbance was measured at 540 nm using a microplate reader (Microquant, Bio-Tek, USA). The percent viability was calculated according to formula given below:

\[
\% \text{ viability of cells} = \left\{ \frac{OD \text{ of treatment}}{OD \text{ of Vehicle control}} \right\} \times 100
\]

1.2.3. Western blot analysis of PSA

LNCaP cells were seeded in culture flasks and incubated for overnight. After 60% confluency cells were treated with agonists, antagonists and respective compounds (testosterone, flutamide compounds $8a$, $8c$ and $9a$). Cells were washed after 24 h of incubation, with ice cold PBS and whole cell lysates of LNCaP prepared in lysis buffer [25 mM tris-HCl, pH 7.6, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% NP-40, 1% protease inhibitor cocktail] were incubated at room temperature for 30 min followed by centrifugation at 14,000 g (4 °C, 25 min). The protein concentration of the supernatant was determined by Bradford protein assay. Samples were boiled for 10 min in denaturing sample buffer (10% glycerol, 1% SDS, 1% β-mercaptoethanol, 10 mM tris-HCl, 0.01% bromophenol blue, pH 6.8), and then 20 µg (protein) of each sample was separated on 10% acrylamide gel and transferred to immobilon-P PVDF membranes (Millipore). After blocking non-specific sites with 5% skimmed milk (in 150 mM NaCl, 10 mM Tris-HCl, pH-7.6) the membranes were probed overnight with antibodies for PSA (1:1000,Sigma-Aldrich), and re-probed with β-actin antibody (1:10,000, Sigma-Aldrich) for loading correction. Subsequently, the blots were washed three times with 0.1% tween 20 in TBS
and incubated with 1:3000 and 1:30,000 dilution of secondary antibody (anti-mouse-IgG-HRP conjugate). After washing in 0.1% tween-20/TBS, substrate solution was added to the membrane, which was incubated for 5 min and exposed at room temperature. The membranes were developed with enhanced chemiluminescence (ECL) kit by following the manufacturer’s protocol.

1.2.4. Reporter Gene Assay by Luciferase Expression

AR-negative Cos7 cells were seeded at a density of 5x10⁴ cells/well into 48-well plates on the day prior to transfection with 500ng of luciferase reporter gene construct pARE-eElB-LUC along with the expression vector for AR, pCR 3.1.AR, using DharmaFECT transfection reagent according to manufacturer’s protocol. At the end of 8 h transfection period, cells were switched to complete medium and thereafter, treated with test compounds and Bicalutamide (AR-antagonist, 3μM) was used as positive control for 24 h. Luciferase activity was determined with luciferase assay systems (Promega, Madison, WI) following the manufacturer’s protocol to detect the AR mediated transcriptional activity. Luciferase activity was normalized for transfection efficiency using pRL-SV40-luc as an internal control.

1.2.5. Calcium mobilization assay

All the analyses was performed with α₁a-adrenergic receptor GPCR cells (Millipore) which was cultured immediately by immersing in a 37 °C water bath in a vial pre-sterilized with 70% ethanol. 1 mL of pre-warmed media component was added to the vial of cells and the volume was raised to 10 mL with media component. Then cell suspension was centrifuged at 190 x g for 4 min, supernatant removed and 5 mL of pre-warmed media was added to re-suspend the cell pellet. 4 x 10⁴ cells per well were seeded overnight in 100 μL using 96-well plates and growth medium. After incubation overnight, the growth medium was removed from the cells and 100 μL/well fluoforte™ dye-loading solution (Enzo life sciences FluoForte Calcium Assay Kit cat. No. ENZ-51017) was added to each well. Thereafter, the culture plates were incubated for 45 min at 37 °C and 15 min at room temperature. α₁-agonist (epinephrine, 20 μM), and antagonists (tamsulosin 1 μM) and the test compounds 8a, 8c and 9a (10 μM) were prepared and the calcium flux assay was run by monitoring the fluorescence at excitation wavelength at 490 nm and emission wavelength of 525 nm, using a fluorescence plate reader (Biotek Flx 800), for 180
sec with agonist addition at 10 sec. Calcium assay was optimized for agonist at one fifth of the final volume and relative fluorescence unit (RFU) values were determined for Ca\(^{2+}\) released in the cells.

### 1.2.6. In vivo experiment

Adult mature Sprague–Dawley rats (weighing 250–280 g), for in vivo study, were procured from the Animal Division of the CSIR-Central Drug Research Institute, Lucknow, India. BPH was induced in rats by a known disease causing agent citral.\(^1\) Animals were erratically divided into five groups, viz. Group I (vehicle control); Group II (Citral 100 mg/kg); Group III (Citral 100 mg/kg + Flutamide 10 mg/kg); Group IV (Citral 100 mg/kg + 8a) and Group V (Citral 100 mg/kg + 9a). Groups II, III, IV, and V were given citral at a dose of 100 mg/kg once daily for 28 days while animals in Group III, IV and V were coadministered with flutamide, 8a and 9a respectively, at 10 mg/kg, treatment beginning from the 8th day of citral treatment and ongoing once daily for remaining 21 days. All compounds were administered orally. At the completion of experiment, animals were sacrificed according to the guidelines of Institutional Animal Ethics Committee, and the weights of prostate, epididymis, seminal vesicles and testis were taken. Prostatic tissues were fixed in 10% formalin solution for histology.

### 1.2.7. Histology

Prostate tissues kept overnight in 10% formalin solution were processed by following the standard protocol. 5.0 µ sections were taken and stained with hematoxyline & eosin and examined under a light microscope (Eclipse 80i, Nikon Corporation, Japan).

### 1.2.8. Pharmacokinetic Studies

The pharmacokinetic studies of 9a was performed in young and healthy male sprague dawley rats weighing 250 ± 25 g obtained from Laboratory Animal Division, CDRI, Lucknow. The animals were housed in plastic cages in standard laboratory conditions with a regular 12 hour day-night cycle. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water were allowed ad libitum. The rats were acclimatized to this environment for at least five days before conducting the experiment. The study was conducted on overnight fasted (12-16 hour) rats (n = 3 per time point). All experiments,
euthanasia and disposal of carcasses were done as per the guidelines of Local Ethics Committee for animal experimentation.

Suspension formulation containing 2.5 mg/mL of 9a was prepared by triturating 9a, gum acacia (1%, w/v) and water (drop wise addition) in a mortar and pestle. A single 10 mg/kg oral dose was given to conscious rats using rat feeding needle. The animals were sacrificed at various predefined times up to 72 h post dose and blood, prostate and hypothalamus were collected. Serum samples were harvested. The hypothalamus of three rats were pooled and homogenized in order to ensure a measurable quantity. All the samples were stored at -80 °C until analysis.

A Shimadzu UFLC pump (LC-20AD) with online degasser (DGU-20A3), an auto-sampler (SIL-HTc) with a temperature-controlled peltier-tray and a triple quadrupole API 4000 Q trap mass spectrometer (Applied Biosystems, Toronto, Canada) was used for analysis. Chromatographic separation was made on a Discovery HS C-18 column (5 μm, 100 x 4.6 mm id) preceded with a guard column (5 μm, 50 x 4.0 mm, id) packed with the same material with mobile phase [85% acetonitrile in aqueous ammonium acetate buffer (0.01 M)] pumped at a flow rate of 0.7 mL/min under isocratic condition. The mobile phase was degassed by ultrasonication for 15 min before use. LC-MS/MS system was equilibrated for approximately 20 min before commencement of analysis. The column oven temperature was 40 °C. Total analysis time was 3 min per sample. The mass spectral analysis was performed in positive ionization mode at 5500 V using multiple reaction monitoring technique to monitor the transitions m/z 455.3 → m/z 220.1 for 9a and m/z 180.1 → m/z 138.2 for phenacetin (internal standard). Data acquisition and quantitation were performed using analyst software (version 1.4.2; AB Sciex, Toronto, Canada). The method was linear over the range of 2 – 200 ng/mL with recovery of >60% and acceptable accuracy and precision.  

1.2.9. Docking studies

The compounds were drawn by using the sketch module of the molecular modeling suite Sybyl 2.1. Tripos force field and Gasteiger–Huckel charges was applied to optimize the geometry of compounds. The energy minimization was done using the Powell method with an energy convergence gradient of 0.001 kcalmol⁻¹. To obtain the probable binding pose the active compounds were subjected to docking program Surf lex-Dock. Surf lex uses an empirical scoring function and a patented search engine to dock ligands into a protein's binding site and is based on
the Hammerhead scoring function and a consensus score that is the linear combination of non-linear functions of protein-ligand atomic surface distances. The interactions include steric, polar, entropic, and solvation terms. In addition, a total score is also generated. The crystal structure of wild type Androgen receptor was retrieved from the protein databank (PDBID: 2AXA), this is co-crystallised with bound ligand designated as S-1. For surflex program the protein receptor was prepared by removing the substructures and extracting ligand. The side chains of the protein structure then were fixed using default settings, water atoms removed, hydrogen added, unknown atom types were assigned and bumps were relaxed. The Kollman-all atom charges were assigned to protein atoms. Protocol was generated using the ligand based mode, residues Leu 704, Asn 705 and Arg 752 were kept as flexible. Docking poses were generated using Geom mode of Surflex-Dock program.
1H NMR of compound 8a

Chemical Formula: $C_{12}H_{14}ClN_3O_3$

13C NMR of compound 8a

Chemical Formula: $C_{12}H_{14}ClN_3O_3$
H NMR of compound 8b

Chemical Formula: C₁₀H₁₃ClN₄O
\(^1\)H NMR of compound 8c

Chemical Formula: C\(_{13}\)H\(_{17}\)ClN\(_2\)O\(_2\)
13C NMR of compound 8c

Chemical Formula: C13H13ClN2O2

1H NMR of compound 8d

Chemical Formula: C13H13ClF3N2O3
$^{13}$C NMR of compound 8d

Chemical Formula: $C_{13}H_{13}ClF_3N_3O$

$^1$H NMR of compound 8e

Chemical Formula: $C_{13}H_{13}ClF_3N_3O_3$
**1H NMR of compound 9a**

Chemical Formula: C$_{22}$H$_{20}$N$_{6}$O$_{5}$

![1H NMR spectrum of compound 9a]
$^{13}$C NMR of compound 9a

$^{1}$H NMR of compound 9b
Chemical Formula: $\text{C}_{23}\text{H}_{29}\text{N}_{5}\text{O}_4$

$^{13}$C NMR of compound 9b
H NMR of compound 9c

Chemical Formula: C_{23}H_{25}N_{2}O_{4}

Chemical Formula: C_{23}H_{25}N_{2}O_{3}
$^{13}$C NMR of compound 9c

Chemical Formula: $C_{20}H_{25}N_7O_3$

$^1$H NMR of compound 9d

Chemical Formula: $C_{21}H_{26}N_2O_3$
$^{13}$C NMR of compound 9d

Chemical Formula: $\text{C}_{21}\text{H}_{26}\text{N}_6\text{O}_3$

$^1$H NMR of compound 9e

Chemical Formula: $\text{C}_{20}\text{H}_{25}\text{N}_7\text{O}_3$
$^1$H NMR of compound 9f

[Chemical formula: $\text{C}_{16}\text{H}_{24}\text{N}_8\text{O}$]

[1H NMR spectrum with peak assignments]

Current Data Parameters
- NAME: S4-4/12
- PROINO: 64
- FZ - Acquisition Parameters
  - DATE: 20110130
  - TIME: 21:45
  - INSTRUMENT: spect
  - OPERATOR: 5
  - T(nm): 1000
  - TD: 60000
  - INSTRUMENT: spect
  - OPERATOR: 5
  - T(nm): 1000

- DATA Acquisition Parameters
  - TR: 0.38
  - DR: 0.035
  - SW: 4000 Hz
  - RES: 10.0
  - R1: 100
  - PD: 3000
  - DE: 10.0
  - T1: 100
  - T2: 1.63

--- CHANNEL F1 ---
- TR: 1000
- DR: 0.1
- SW: 10000 Hz
- RES: 1000 Hz
- R1: 100
- PD: 3000
- DE: 10.0
- T1: 100
- T2: 1.63

--- Processing Parameters ---
- SF: 265.300000 Hz
- WRF: 0.0
- AM: 0 Hz
- PM: 0 Hz
- PC: 0.0
$^{13}$C NMR of compound 9f

Chemical Formula: $C_{18}H_{24}N_8O$

$^1H$ NMR of compound 9g
$^{13}$C NMR of compound 9g

Chemical Formula: $C_{21}H_{28}N_6O_2$
\( ^1H \) NMR of compound 9h

Chemical Formula: \( C_{19}H_{26}N_7O \)

\( ^13C \) NMR of compound 9h

Chemical Formula: \( C_{19}H_{26}N_7O \)
**1H NMR of compound 9i**

![1H NMR spectrum of compound 9i]

Chemical Formula: $C_{23}H_{29}N_5O_4$

**13C NMR of compound 9i**

![13C NMR spectrum of compound 9i]

Chemical Formula: $C_{23}H_{29}N_5O_4$
**1H NMR of compound 9j**

Chemical Formula: $C_{24}H_{24}N_4O_3$

**13C NMR of compound 9j**

Chemical Formula: $C_{24}H_{24}N_4O_3$
\[ ^1H \text{NMR of compound 9k} \]

Chemical Formula: \( C_{22}H_{29}N_5O_2 \)

\[ \text{\textbf{\textit{\textsuperscript{13}C NMR of compound 9k}}} \]

Chemical Formula: \( C_{22}H_{29}N_5O_2 \)
**1H NMR of compound 9m**

Chemical Formula: $C_{24}H_{24}F_6N_6O_5$

---

**13C NMR of compound 9m**

Chemical Formula: $C_{24}H_{24}F_6N_6O_5$
$^{1}H$ NMR of compound 9n

Chemical Formula: $C_{24}H_{24}F_{6}N_{6}O_{5}$

$^{13}C$ NMR of compound 9n

Chemical Formula: $C_{24}H_{24}F_{6}N_{6}O_{5}$
$^1$H NMR of compound 11a

Chemical Formula: $C_{16}H_{16}N_4O_4$

$^{13}$C NMR of compound 11a

Chemical Formula: $C_{16}H_{16}N_4O_4$
¹H NMR of compound 11b

Chemical Formula: C₁₇H₁₆N₄O₆

³¹C NMR of compound 11b

Chemical Formula: C₁₇H₁₆N₄O₆
\( ^1H \) NMR of compound 12a

Chemical Formula: \( C_{21}H_{23}F_3N_3O_3 \)

\[ \begin{align*}
  \text{NMR Parameters:} \\
  \text{Current Data Parameters} \\
  \text{NAME: MOB-101-27-1H} \\
  \text{EXPERI: 370} \\
  \text{PROCNO: 1} \\
  \text{F2 - Acquisition Parameters} \\
  \text{Date: 2015/07/02} \\
  \text{Time: 6.46} \\
  \text{INSTRUM: spect} \\
  \text{PULPROG: 5 mm PABBO BB} \\
  \text{TD: 65526} \\
  \text{SOLVENT: CDCl3} \\
  \text{RE: 0} \\
  \text{SW1: 9012.620 Hz} \\
  \text{FIDRES: 0.122996 Hz} \\
  \text{AQ: 4.0934465 sec} \\
  \text{RG: 80.54} \\
  \text{DW: 62.400 usec} \\
  \text{DE: 5.50 usec} \\
  \text{TE: 296.5 K} \\
  \text{D1: 1.000000000 sec} \\
  \text{T00: 1} \\
  \text{CHANNEL 11} \\
  \text{SF: 400.1602/12 MHz} \\
  \text{NUC: 1H} \\
  \text{F: 13.80 usec} \\
  \text{PLW1: 13.00000036 W} \\
  \text{F2 - Processing parameters} \\
  \text{SF: 400.1602/00 MHz} \\
  \text{FW: no} \\
  \text{SB: 0 Hz} \\
  \text{SB: 0 Hz} \\
  \text{PC: 1.00} \\
\end{align*} \]

\( ^{13}C \) NMR of compound 12a

Chemical Formula: \( C_{21}H_{23}F_3N_3O_3 \)

\[ \begin{align*}
  \text{NMR Parameters:} \\
  \text{Current Data Parameters} \\
  \text{NAME: MOB-101-27-13C} \\
  \text{EXPERI: 349} \\
  \text{PROCNO: 1} \\
  \text{F2 - Acquisition Parameters} \\
  \text{Date: 2015/07/04} \\
  \text{Time: 3.32} \\
  \text{INSTRUM: spect} \\
  \text{PULPROG: 5 mm PABBO BB} \\
  \text{TD: 65526} \\
  \text{SOLVENT: CDCl3} \\
  \text{RE: 122} \\
  \text{SW1: 34629.461 Hz} \\
  \text{FIDRES: 0.000050 Hz} \\
  \text{AQ: 21.46 usec} \\
  \text{RG: 0.000000 sec} \\
  \text{DE: 0.000000 sec} \\
  \text{TE: 310.5 K} \\
  \text{D1: 200.000000000 sec} \\
  \text{T00: 1} \\
\end{align*} \]
$^{1}H$ NMR of compound 12b

Chemical Formula: $C_{21}H_{25}F_{3}N_{6}O_{2}$

$^{13}C$ NMR of compound 12b

Chemical Formula: $C_{21}H_{25}F_{3}N_{6}O_{2}$
$^1$H NMR of compound 12c

Chemical Formula: $C_{19}H_{24}N_6O$

$^{13}$C NMR of compound 12c

Chemical Formula: $C_{19}H_{24}N_6O$
**1H NMR of compound 12d**

![1H NMR Spectrum](image1)

Chemical Formula: $C_{20}H_{24}N_6O_3$

**13C NMR of compound 12d**

![13C NMR Spectrum](image2)

Chemical Formula: $C_{20}H_{24}N_6O_3$
$^{1}H$ NMR of compound 12e

![$^{1}H$ NMR spectrum of compound 12e](image)

Chemical Formula: $C_{2}H_{2}N_{6}O_{5}$

$^{13}C$ NMR of compound 12e

![$^{13}C$ NMR spectrum of compound 12e](image)

Chemical Formula: $C_{2}H_{2}N_{6}O_{5}$
$^1$H NMR of compound 12f

Chemical Formula: $C_{22}H_{23}F_3N_6O_5$

$^{13}$C NMR of compound 12f

Chemical Formula: $C_{22}H_{23}F_3N_6O_5$
$^1$H NMR of compound 12g

Chemical Formula: \( \text{C}_{22}\text{H}_{26}\text{F}_{3}\text{N}_{6}\text{O}_{5} \)

$^{13}$C NMR of compound 12g

Chemical Formula: \( \text{C}_{22}\text{H}_{26}\text{F}_{3}\text{N}_{6}\text{O}_{5} \)
HRMS of compound 8a

Chemical Formula: C_{12}H_{10}ClN_{2}O_{3} + H
Exact Mass: 284.0802

HRMS of compound 8b

Chemical Formula: C_{13}H_{12}ClN_{2}O + H
Exact Mass: 241.0856
HRMS of compound 8c

Chemical Formula: C_{13}H_{17}ClN_{2}O_{2} + H
Exact Mass: 269.1057

HRMS of compound 8d

Chemical Formula: C_{13}H_{13}ClF_{3}N_{2}O_{3} + H
Exact Mass: 352.0676
HRMS of compound 9a

Chemical Formula: C$_2$H$_2$N$_6$O$_5$ + H
Exact Mass: 455.2043

HRMS of compound 9b

Chemical Formula: C$_2$H$_2$N$_6$O$_5$ + H
Exact Mass: 440.2298
HRMS of compound 9c

Chemical Formula: C_{20}H_{25}N_{7}O_{3} + H
Exact Mass: 412.2097

HRMS of compound 9d

Chemical Formula: C_{21}H_{26}N_{6}O_{3} + H
Exact Mass: 411.2145
ESI-MS of compound 9e

HRMS of compound 9f
HRMS of compound 9g

Chemical Formula: C_{21}H_{28}N_{6}O_{2} + H
Exact Mass: 397.2352

HRMS of compound 9h

Chemical Formula: C_{19}H_{25}N_{7}O + H
Exact Mass: 368.2199
HRMS of compound 9i

Chemical Formula: C₂₃H₂₉N₇O₄ + H
Exact Mass: 440.2298

HRMS of compound 9j

Chemical Formula: C₂₄H₂₉N₇O₄ + H
Exact Mass: 425.2553
HRMS of compound 9k

Chemical Formula: C_{21}H_{28}N_{6}O_{2} + H
Exact Mass: 396.2400

HRMS of compound 9l

Chemical Formula: C_{22}H_{29}N_{6}O_{2} + H
Exact Mass: 397.2352
HRMS of compound 9m

Chemical Formula: $C_2H_2F_6N_6O_5 + H$
Exact Mass: 591.1791

HRMS of compound 9n

Chemical Formula: $C_2H_2F_6N_6O_5 + H$
Exact Mass: 591.1791
HRMS of compound 12a

Chemical Formula: C₂H₂F₃N₂O₃ + H
Exact Mass: 465.1862

HRMS of compound 12b

Chemical Formula: C₂H₂F₃N₂O₃ + H
Exact Mass: 465.1862
HRMS of compound 12c

Chemical Formula: C\textsubscript{14}H\textsubscript{12}N\textsubscript{6}O + H

Exact Mass: 353.2090

HRMS of compound 12d

Chemical Formula: C\textsubscript{20}H\textsubscript{17}N\textsubscript{6}O\textsubscript{3} + H

Exact Mass: 397.1988
HRMS of compound 12e

Chemical Formula: C_{22}H_{26}F_{3}N_{6}O_{5} + H

Exact Mass: 509.1760

HRMS of compound 12f

Chemical Formula: C_{22}H_{26}F_{3}N_{6}O_{5} + H

Exact Mass: 509.1760
HRMS of compound 12g

Chemical Formula: C_{22}H_{23}F_{3}N_{6}O_{5} + H
Exact Mass: 509.1760
Sample Conc. 1000 ng/mL  
Mobile Phase: ACN: MeOH: AAB (10 mM) :: 50:10:40  
Run Time: 10 min  
$\lambda_{\text{max}}$: 387 nm  
Flow rate: 1 mL/min  
Column: Discovery HS C-18 10 cm (Reverse phase)  
Retention time: 4.5  
Purity: 97%

**Figure 1:** Representative chromatogram of 9a

**REFERENCES**


