Electronic Supplementary Information

Pd-Mediated new synthesis of pyrroles: Their evalution as potential inhibitors of phosphodiesterase 4

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Experimental

Chemistry

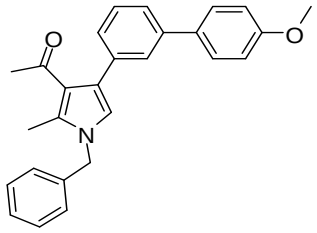
General methods: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were recodred in $CDCl_3$ solution by using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT- IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a Agilent 6430 series Triple Quard LC-MS / MS spectrometer. High-resolution mass spectra (HRMS) were recorded using a Waters LCT Premier XE instrument. Melting points (mp) were by using Buchi B-540 melting point appratus.

MCR based on Suzuki coupling: A typical procedure

To a stirring solution of benzylamine (1.5 mmol), acetylacetone (1.0 mmol), 3-bromobenzaldehyde (1.0 mmol) and nitromethane (3.0 mL) was added $(PPh_3)_2PdCl_2$ (0.1 mol%) at room temp. The mixture was then stirred at 80-85 ºC and the progress was monitored by TLC. After completion of step 1 (indicated by TLC) the mixture was cooled to room temp. To this was added 1,4-dioxane (4.0 mL), water (1.0 mL), arylboronic acid (1.5 mmol) and K_2CO_3 (2.0 mmol) with stirring. The mixture was then stirred at 80-85 ºC for the time indicated in Table 2. After completion of the reaction the mixture was cooled to room temp, diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layers were collected, combined, washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using 1:10 - 2:8 ethyl acetate: petroleum ether to afford the desired compound.

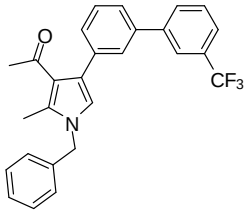
Spectral data of compound 6

1-(1-benzyl-4-(4'-methoxy-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6a)



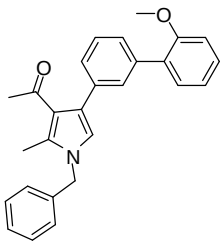
White solid (336 mg, 85%); mp 125-127 °C; IR (neat) 3436, 1879, 1644, 1600, 1506, 1480, 1249, 1181 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 3.85 (s, 3H), 5.07 (s, 2H), 6.58 (s, 1H), 6.96 (d, *J* = 4.8 Hz, 2H), 7.10 (d, *J* = 7.2 Hz, 2H), 7.25-7.55 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 55.3, 114.1, 120.1, 122.0, 125.0, 126.6, 127.6, 128.1, 133.4, 135.2, 136.5, 140.6, 159.1, 197.5; HRMS: *m/z* calcd for $C_{27}H_{26}NO_2$ (M + 1) 396.1885; found 396.1966.

1-(1-benzyl-2-methyl-4-(3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-1H-pyrrol-3-yl)ethanone (6b)



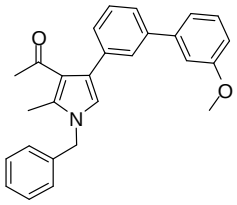
White solid (359 mg, 83%); mp 90-92 °C; IR (neat) 3272, 3110, 2929, 1879, 1644, 1604, 1328, 1269 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 5.08 (s, 2H), 6.60 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.26-7.57 (m, 7H), 7.67-7.72 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 120.3, 122.0, 125.4, 126.6, 127.3, 128.1, 129.1, 135.3, 137.0, 139.6, 144.4, 197.2; HRMS: *m/z* calcd for $C_{27}H_{23}F_3NO$ (M + 1) 434.1653; found 434.1736.

1-(1-benzyl-4-(2'-methoxy-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6c)



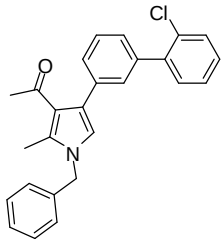
White solid (320 mg, 81%); mp 126-128 °C; IR (neat) 3077, 2928, 1879, 1644, 1506, 1412, 1249, 1027 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 3.85 (s, 3H), 5.07 (s, 2H), 6.58 (s, 1H), 6.98 (d, *J* = 6.8 Hz, 2H), 7.10 (d, *J* = 6.8 Hz, 2H), 7.25-7.41 (m, 5H), 7.46-7.56 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.2, 55.2, 114.1, 120.1, 122.0, 124.9, 126.6, 127.6, 128.0, 133.3, 135.1, 136.4, 140.6, 159.1, 197.4; HRMS: *m/z* calcd for $C_{27}H_{26}NO_2$ (M + 1) 396.1885; found 396.1963.

1-(1-benzyl-4-(3'-methoxy-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6d)



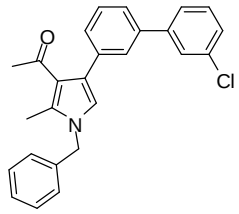
White solid (328 mg, 83%); mp 127-129 °C; IR (neat) 3112, 2929, 1879, 1644, 1600, 1412, 1249, 1181 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 3.85 (s, 3H), 5.07 (s, 2H), 6.58 (s, 1H), 6.97 (d, *J* = 4.8 Hz, 2H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.25-7.55 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 55.3, 114.1, 120.1, 122.0, 125.0, 126.6, 127.6, 128.1, 133.4, 135.1, 136.5, 140.6, 159.1, 197.5; HRMS: *m/z* calcd for $C_{27}H_{26}NO_2$ (M + 1) 396.1885; found 396.1963.

1-(1-benzyl-4-(2'-chloro-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6e)



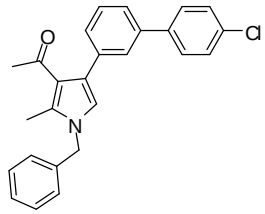
White solid (315 mg, 79%); mp 116.5-118.5 °C; IR (neat) 3274, 3025, 1800, 1628, 1604, 1506, 1245, 704 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.11 (s, 3H), 2.44 (s, 3H), 5.06 (s, 2H), 6.58 (s, 1H), 7.09 (d, *J* = 6.8 Hz, 2H), 7.26-7.40 (m, 8H), 7.41-7.47 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.2, 120.2, 122.0, 125.5, 126.6, 127.6, 128.4, 129.8, 130.3, 131.2, 132.4, 135.1, 136.0, 139.3, 140.2, 197.5; HRMS: *m/z* calcd for $C_{26}H_{23}N_2OCl$ (M + 1) 400.1390; found 400.1463.

1-(1-benzyl-4-(3'-chloro-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6f)



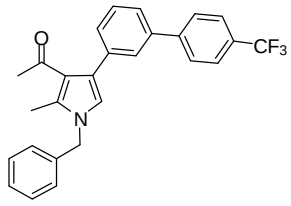
White solid (311 mg, 78%); mp 117-119 °C; IR (neat) 3273, 3025, 2924, 1800, 1628, 1592, 1506, 1245, 704 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.45 (s, 3H), 5.07 (s, 2H), 6.59 (s, 1H), 7.11 (d, *J* = 6.8 Hz, 2H), 7.26-7.58 (m, 11H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 120.2, 122.0, 125.5, 126.6, 127.7, 128.4, 129.9, 130.3, 131.3, 132.4, 135.1, 136.0, 139.3, 140.2, 197.5; HRMS: *m/z* calcd for $C_{26}H_{23}N_2OCl$ (M + 1) 400.1390; found 400.1466.

1-(1-benzyl-4-(4'-chloro-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6g)



White solid (319 mg, 80%); mp 115-117 °C; IR (neat) 3111, 2955, 1890, 1643, 1544, 1245, 706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 5.07 (s, 2H), 6.59 (s, 1H), 7.10 (d, *J* = 6.8 Hz, 2H), 7.26-7.54 (m, 11H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.2, 120.2, 122.0, 125.5, 126.6, 127.6, 128.4, 129.8, 130.3, 131.2, 132.4, 135.1, 136.0, 139.3, 140.2, 197.5; HRMS: *m/z* calcd for $C_{26}H_{23}N_2OCl$ (M + 1) 400.1390; found 400.1475.

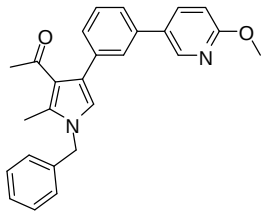
1-(1-benzyl-2-methyl-4-(4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-1H-pyrrol-3-yl)ethanone (6h)



White solid (338 mg, 78%); mp 92-94 °C; IR (neat) 3110, 2929, 1810, 1645, 1603, 1506, 1205, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 5.07 (s, 2H), 6.60 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.25-7.38 (m, 7H), 7.44-7.72 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ

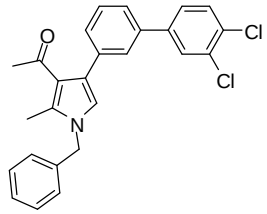
11.6, 31.1, 50.3, 120.2, 122.0, 125.4, 126.6, 127.4, 128.1, 129.1, 135.3, 136.4, 137.0, 139.6, 144.4, 197.2; HRMS: *m/z* calcd for $C_{27}H_{23}F_3NO$ (M + 1) 434.1653; found 434.1720.

1-(1-benzyl-4-(3-(6-methoxypyridin-3-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (6i)



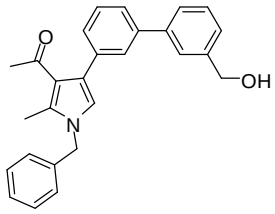
White solid (297 mg, 75%); mp 97-98 °C; IR (neat) 3061, 2945, 1951, 1646, 1459, 1291, 1022 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H), 2.45 (s, 3H), 3.98 (s, 3H), 5.07 (s, 2H), 6.59 (s, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 6.4 Hz, 2H), 7.26-7.49 (m, 8H), 7.8 (d, *J* = 6.0 Hz, 1H), 8.4 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 53.5, 110.7, 120.2, 122.0, 124.9, 125.5, 126.6, 127.5, 128.2, 129.8, 135.2, 136.4, 137.4, 144.9, 163.6, 197.3; HRMS: *m/z* calcd for $C_{26}H_{25}N_2O_2$ (M + 1) 397.1838; found 397.1917.

1-(1-benzyl-4-(3',4'-dichloro-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6j)



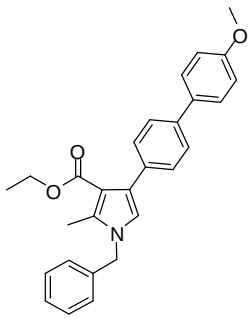
White solid (346 mg, 80%); mp 105-107 °C; IR (neat) 3111, 3074, 2926, 1886, 1644, 1556, 1204, 1135, 705 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.45 (s, 3H), 5.07 (s, 2H), 6.59 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.26-7.37 (m, 4H), 7.41-7.50 (m, 5H), 7.68 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 120.2, 122.0, 125.2, 126.3, 127.7, 128.8, 129.1, 130.6, 131.5, 132.8, 136.4, 137.1, 138.6, 140.9, 197.2; HRMS: *m/z* calcd for $C_{26}H_{22}Cl_2NO$ (M + 1) 434.1000; found 434.1099.

1-(1-benzyl-4-(3'-(hydroxymethyl)-[1,1'-biphenyl]-3-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (6k)



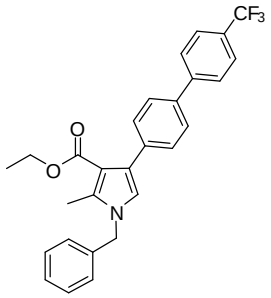
Brown sticky liquid (316 mg, 80%); IR (neat) 3400, 3029, 1644, 1601, 1508, 1415, 1266, 1180, 1028 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.45 (s, 3H), 4.76 (s, 3H), 5.07 (s, 2H), 6.59 (s, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 7.25-7.36, (m, 5H), 7.41-7.54 (m, 2H), 7.57-7.61(m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 31.1, 50.3, 65.3, 120.2, 122.0, 125.4, 126.4, 127.8, 128.0, 129.0, 135.3, 136.4, 140.8, 14.2, 197.6; HRMS: *m/z* calcd for $C_{27}H_{26}NO_2$ (M + 1) 396.1885; found 396.1958.

Ethyl 1-benzyl-4-(4'-methoxy-[1,1'-biphenyl]-4-yl)-2-methyl-1H-pyrrole-3-carboxylate (6l)



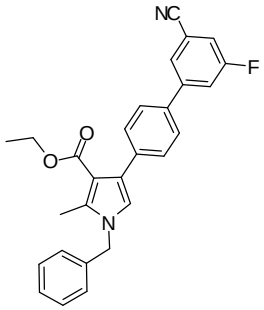
Off-White solid (336 mg, 79%); mp 117-119 °C; IR (neat) 2907, 1686, 1605, 1518, 1413, 1285, 1184, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (t, *J* = 7.2 Hz, 3H), 2.48 (s, 3H), 3.85 (s, 3H), 4.21 (q, *J* = 7.2 Hz, 2H), 5.08 (s, 2H), 6.62 (s, 1H), 6.98 (d, *J* = 2.0 Hz, 2H), 7.09 (d, *J* = 7.2 Hz, 2H), 7.26-7.57 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 14.1, 50.5, 55.2, 59.4, 110.9, 114.1, 120.4, 125.7, 126.5, 127.7, 128.8, 129.5, 130.4, 133.7, 134.2, 135.1, 136.4, 138.4, 158.9, 165.8; HRMS: *m/z* calcd for $C_{28}H_{28}NO_3$ (M + 1) 426.1991; found 426.2087.

Ethyl 1-benzyl-2-methyl-4-(4'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-1H-pyrrole-3-carboxylate (6m)



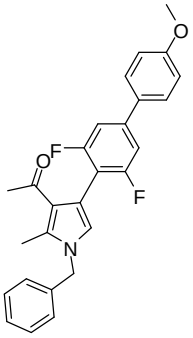
White solid (347 mg, 75%); mp 107-109 °C; IR (neat) 3444, 3030, 2911, 1686, 1606, 1329, 1285, 1073 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.17 (t, *J* = 7.2 Hz, 3H), 2.49 (s, 3H), 4.22 (q, *J* = 7.2 Hz, 2H), 5.09 (s, 2H), 6.64 (s, 1H), 7.09 (d, *J* = 7.2 Hz, 2H), 7.25-7.37 (m, 3H) 7.48-7.73 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 14.1, 50.5, 59.5, 110.9, 120.6, 125.5, 126.3, 127.1, 135.8, 136.5, 137.3, 165.7; HRMS: *m/z* calcd for $C_{28}H_{25}F_3NO_2$ (M + 1) 464.1759; found 464.1834.

Ethyl 1-benzyl-4-(3'-cyano-5'-fluoro-[1,1'-biphenyl]-4-yl)-2-methyl-1H-pyrrole-3-carboxylate (6n)



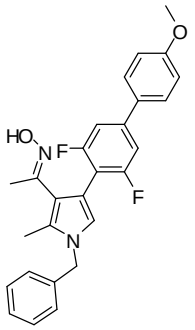
Light brown sticky liquid (360 mg, 82%); IR (neat) 3020, 2400, 2235, 1686, 1607, 1591, 1420, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.2 (t, *J* = 8.0 Hz, 3H), 2.49 (s, 3H), 4.21 (q, 2H), 5.09 (s, 2H), 6.65 (s, 1H), 6.91 (s, 1H), 7.10 (d, *J* = 7.2 Hz, 2H), 7.29-7.37 (m, 3H), 7.50-7.55 (m, 5H), 7.69 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 14.1, 50.6, 59.8, 108.3, 110.5, 113.9, 115.3, 117.0, 118.5, 120.8, 125.3, 126.1, 127.9, 128.9, 130.0, 135.2, 136.4, 145.0, 158.5, 161.3, 163.8, 166.1; HRMS: *m/z* calcd for $C_{28}H_{24}FN_2O_2$ (M + 1) 439.1744; found 439.1837.

1-(1-benzyl-4-(3,5-difluoro-4'-methoxy-[1,1'-biphenyl]-4-yl)-2-methyl-1H-pyrrol-3-yl)ethanone (7)



White solid (345 mg, 80%); mp 144-147 °C; IR (neat) 3685, 3584, 3019,2936, 2400, 1885, 1650, 1584, 1519, 1417, 1215, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.14 (s, 3H), 2.47 (s, 3H), 3.86 (s, 3H), 5.10 (s, 2H), 6.67 (s, 1H), 7.00 (d, *J* = 2.0 Hz, 2H), 7.10 (d, *J* = 7.6 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 7.25-7.38 (m, 3H), 7.54 (d, *J* = 78.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8, 29.6, 50.4, 55.3, 109.0, 110.0, 114.4, 122.1, 126.6, 127.9, 128.9, 131.0, 135.6, 136.3, 141.8, 159.4, 161.8, 195.9; HRMS: *m/z* calcd for $C_{27}H_{24}NO_2F_2$ (M + 1) 432.1697; found 432.1816.

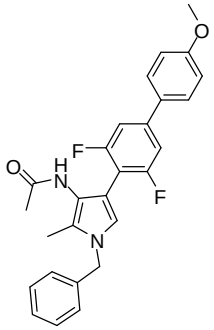
Preparation of (*E*)-1-(1-benzyl-4-(3,5-difluoro-4'-methoxy-[1,1'-biphenyl]-4-yl)-2-methyl-1*H*-pyrrol-3-yl)ethanone oxime (8)



A mixture of compound **7** (1.0 mmol), hydroxylamine hydrochloride (1.5 mmol) and NaOAc (1.5 mmol) in 1:1 ethanol- water (6.0 mL) was stirred for 15 min at room temp and then at 80-85 ºC for 3h (the reaction was monitored by TLC). After completion of the reaction the mixture was completely evaporated under vacuum. The residue was treated with ethyl acetate (15 mL) and washed with water (2 x 10 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography to give the desired product as a white solid (388 mg, 0.87 mmol, 87%); mp 184-186 °C; IR (neat) 3585, 3307, 3019, 2926, 2433, 1885, 1609, 1584, 1518, 1434, 1215, 1182, 1023 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.98 (s, 3H), 2.27 (s, 3H), 3.85 (s, 3H), 5.08 (s, 2H), 6.81 (s, 1H), 6.98 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 7.25-7.36 (m, 5H),

7.52 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.9, 15.2, 29.6, 50.7, 55.03, 108.6, 109.0, 111.0, 114.3, 118.3, 121.8, 126.6, 127.5, 128.2, 131.2, 137.7, 140.5, 154.0, 159.1, 161.6; HRMS: *m/z* calcd for $C_{27}H_{25}N_2O_2F_2$ (M + 1) 447.1806; found 447.1877.

Preparation of *N*-(1-benzyl-4-(3,5-difluoro-4'-methoxy-[1,1'-biphenyl]-4-yl)-2-methyl-1H-pyrrol-3-yl)acetamide (9)



A mixture of compound **8** (2.0 mmol) and cyanuric chloride **(**0.1 mmol) in dry MeCN (4.0 mL) was refluxed for 3h. After completion of the reaction, the mixture was quenched with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3 x 5 mL). The organic layers were collected, combined, dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The crude product was purified by column chromatography on silica gel to give the desired product as a white solid (379 mg, 85%); mp 80-83 °C; IR (neat) 3722, 2924, 2849, 1811, 1607, 1557, 1495, 1454, 1253, 1219, 1179 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.18 (s, 3H), 3.85 (s, 3H), 5.08 (d, *J* = 10.4 Hz, 11.6 Hz, 2H), 6.82 (d, *J* = 10.0 Hz, 1H), 6.96-7.17 (m, 6H), 7.25-7.37 (m, 3H), 7.49-7.53 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.2, 14.0, 23.3, 51.2, 55.3, 109.2, 114.3, 116.4, 118.6, 119.9, 120.1, 125.8, 126.3, 127.4, 128.8, 131.1, 137.0, 159.8, 169.1, 174.5; HRMS: *m/z* calcd for $C_{27}H_{25}N_2O_2F_2$ (M + 1) 447.1806; found 447.1899.

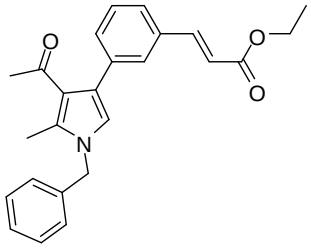
MCR based on Heck coupling: A typical procedure

To a stirring solution of benzylamine (1.5 mmol), acetylacetone (1.0 mmol), 3-bromobenzaldehyde (1.0 mmol) and nitromethane (3.0 mL) was added $(PPh_3)_2PdCl_2$ (0.1 mol%) at room temp. The mixture was then stirred at 80-85 ºC and the progress was monitored by TLC. After completion of step 1 (indicated by TLC) the mixture was cooled to room temp. To this was added DMF (5.0 mL), an alkene (1.5 mmol) and Et_3N (3.0 mmol) with stirring. The mixture was

then stirred at 80-85 ºC for 5-6 h. After completion of the reaction the mixture was cooled to room temp, diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layers were collected, combined, washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using 1:9- 2:8 ethyl acetate: petroleum ether to afford the desired compound.

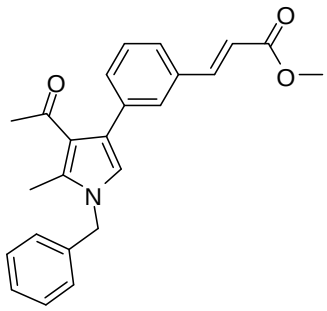
Spectral data of compound 10

(*E*)-ethyl 3-(3-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)phenyl)acrylate (10a)



Broun sticky liquid (290 mg, 75%); IR (neat) 3683, 3019, 2930, 2856, 1706, 1639, 1508,1455, 1368, 1271, 1215, 1181 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, *J* = 7.2 Hz, 3H), 2.04 (s, 3H), 2.44 (s, 3H), 4.27 (q, *J* = 7.2 Hz, 2H), 5.07 (s, 2H), 6.45 (d, *J* = 15.6 Hz, 1H), 6.56 (s, 1H), 7.10 (d, *J* = 6.8 Hz, 2H), 7.26-7.49 (m, 7H), 7.71 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 14.2, 29.6, 50.3, 60.4, 118.5, 120.2, 121.9, 124.9, 126.2, 127.9, 128.6, 129.0, 131.2, 134.3, 135.4, 136.3, 137.0, 144.3, 166.9, 197.1; HRMS: *m/z* calcd for $C_{25}H_{26}NO_3$ (M + 1) 388.1834; found 388.1924.

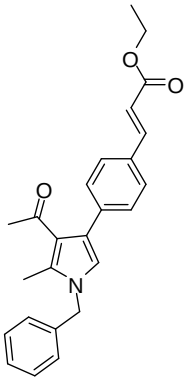
(*E*)-methyl 3-(3-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)phenyl)acrylate (10b)



Pale yellow sticky liquid (269 mg, 72%); IR (neat) 3684, 3063, 3016, 2952, 2854, 1949, 1715, 1638, 1603, 1546, 1436, 1389, 1204 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.44 (s,

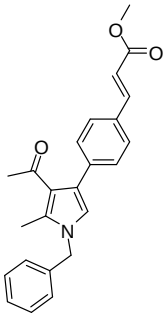
6H), 3.80 (s, 3H), 5.07 (s, 2H), 6.47 (d, *J* = 16.0 Hz, 1H), 6.56 (s, 1H), 7.10 (d, *J* = 7.2 Hz, 2H), 7.26-7.49(m, 7H), 7.72 (d, *J* = 16.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 29.6, 50.3, 51.6, 118.0, 120.2, 121.9, 124.9, 126.2, 127.8, 128.3, 129.0, 131.2, 134.3, 135.4, 136.3, 137.0, 144.6, 167.3, 197.1; HRMS: *m/z* calcd for $C_{24}H_{24}NO_3$ (M + 1) 374.1678; found 374.1740.

(*E*)-ethyl 3-(4-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)phenyl)acrylate (10c)



Light brown sticky liquid (317 mg, 82%); IR (neat) 3675, 3065, 2927, 2455, 1951, 1804, 1708, 1635, 1548, 1498, 1310, 1205, 1177, 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35 (t, *J* = 7.6 Hz, 3H), 2.08 (s, 3H), 2.42 (s, 3H), 4.29 (q, *J* = 7.6 Hz, 2H), 5.06 (s, 2H), 6.46 (d, *J* = 16.0 Hz, 1H), 6.57 (s, 1H), 7.09 (d, *J* = 6.8 Hz, 2H), 7.26-7.36 (m, 5H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.71 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.4, 14.2, 29.6, 50.3, 60.4, 116.6, 117.7, 120.3, 124.9, 126.1, 127.8, 128.9, 129.5, 132.7, 135.3, 136.3, 139.0, 144.1, 148.4, 167.0, 197.3; HRMS: *m/z* calcd for $C_{25}H_{26}NO_3$ (M + 1) 388.1834; found 388.1917.

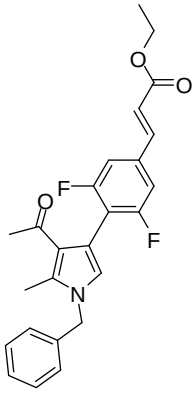
(*E*)-methyl 3-(4-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)phenyl)acrylate (10d)



Light brown solid (302 mg, 81%); mp 55-57 °C; IR (neat) 3684, 3584, 3019, 2927, 2434, 1914, 1709, 1636, 1547, 1436, 1354, 1278, 1175, 1077 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.08 (s, 3H), 2.42 (s, 6H), 3.81 (s, 3H), 5.06 (s, 2H), 6.46 (d, *J* = 16.0 Hz, 1H), 6.57(s, 1H), 7.09(d, *J* = 7.2 Hz, 2H), 7.26-7.37 (m, 5H), 7.52 (d, *J* = 7.6 Hz, 2H), 7.72 (d, *J* = 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 29.6, 50.3, 51.6, 117.2, 120.3, 122.0, 125.0, 126.6, 127.9, 128.0,

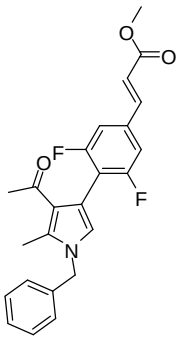
129.5, 132.6, 135.3, 136.3, 138.4, 144.5, 167.5, 197.4; HRMS: *m/z* calcd for $C_{24}H_{24}NO_3$ (M + 1) 374.1678; found 374.1742.

(*E*)-ethyl 3-(4-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)-3,5-difluorophenyl)acrylate (10e)



Yellow solid (330 mg, 78%); mp 127-130 °C; IR (neat) 3683, 3018, 2926, 2401, 1711, 1645, 1562, 1427, 1353, 1266, 1180, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.36 (t, *J* = 7.2 Hz, 3H), 2.13 (s, 3H), 2.45 (s, 3H), 4.30 (q, *J* = 7.2 Hz, 2H), 5.09 (s, 2H), 6.44 (d, *J* = 16.0 Hz, 1H), 6.68 (s, 1H), 7.07-7.11 (m, 4H), 7.26-7.37 (m, 3H), 7.60 (d, *J* = 15.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7, 14.0, 29.4, 50.4, 60.6, 109.3, 110.4, 115.2, 120.3, 122.3, 126.4, 127.8, 128.8, 135.1, 136.1, 141.8, 161.5, 166.2, 195.4; HRMS: *m/z* calcd for $C_{25}H_{224}F_2NO_3$ (M + 1) 424.1646; found 424.1732.

(*E*)-methyl 3-(4-(4-acetyl-1-benzyl-5-methyl-1H-pyrrol-3-yl)-3,5-difluorophenyl)acrylate (10f)



Light brown solid (307 mg, 75%); mp 135-138 °C; IR (neat) 3685, 3584, 3020, 2433, 1884, 1716, 1643, 1521, 1476, 1334, 1215, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.1 (s, 3H), 2.45 (s, 6H), 3.82 (s, 3H), 5.09 (s, 2H), 6.45 (d, *J* = 16.4 Hz, 1H), 6.68 (s, 1H), 7.07-7.11 (m, 4H), 7.26-7.35(m, 3H), 7.61 (d, *J* = 16.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7, 29.5, 50.5,

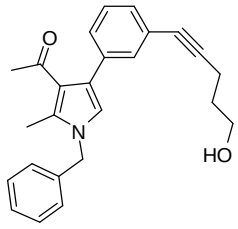
51.8, 109.4, 110.7, 115.3, 119.6, 122.3, 126.5, 127.9, 128.3, 134.9, 135.0, 136.1, 142.2, 161.6, 166.7, 195.5; HRMS: *m/z* calcd for $C_{24}H_{22}F_2NO_3$ (M + 1) 410.1489; found 410.1581.

MCR based on Sonogashira coupling: A typical procedure

To a stirring solution of benzylamine (1.5 mmol), acetylacetone (1.0 mmol), 3-bromobenzaldehyde (1.0 mmol) and nitromethane (3.0 mL) was added $(PPh_3)_2PdCl_2$ (0.1 mol%) at room temp. The mixture was then stirred at 80-85 ºC and the progress was monitored by TLC. After completion of step 1 (indicated by TLC) the mixture was cooled to room temp. To this was added DMF (6.0 mL), a terminal alkyne (1.5 mmol), CuI (0.1 mmol) and Et_3N (3.0 mmol) with stirring. The mixture was then stirred at 80-85 ºC for 5-6 h. After completion of the reaction the mixture was cooled to room temp, diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layers were collected, combined, washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography on silica gel using 1:9 - 2:8 ethyl acetate: petroleum ether to afford the desired compound.

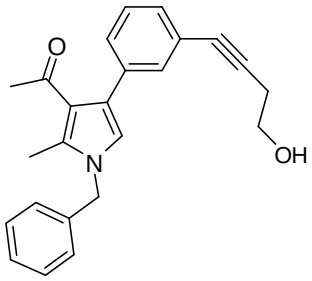
Spectral data of compound 11 and 12:

1-(1-benzyl-4-(3-(5-hydroxypent-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (11a)



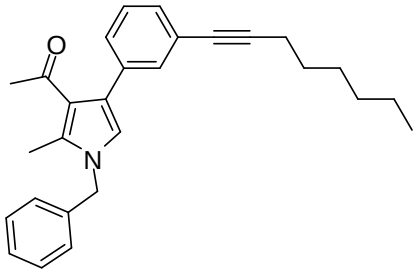
Light brown sticky liquid (301 mg, 81%); IR (neat) 3685, 3020, 2928, 2235,1686, 1607, 1591,1528, 1420, 1332, 1287, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.83 (m, 3H), 2.04 (s, 3H), 2.43 (s, 3H), 2.56 (t, *J* = 6.8 Hz, 2H), 3.84 (t, *J* = 6.4 Hz, 2H), 5.05(s, 2H), 6.53 (s, 1H), 7.08 (d, *J* = 6.8 Hz, 2H), 7.21-7.37 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ11.5, 16.0, 29.6, 31.1, 50.3, 61.8, 80.9, 89.4, 120.2, 121.9, 123.6, 125.0, 126.6, 127.8, 128.0, 129.7, 132.2, 135.2, 136.4, 197.4; HRMS: *m/z* calcd for $C_{25}H_{26}NO_2$ (M + 1) 372.1885; found 372.1953.

1-(1-benzyl-4-(3-(4-hydroxybut-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (11b)



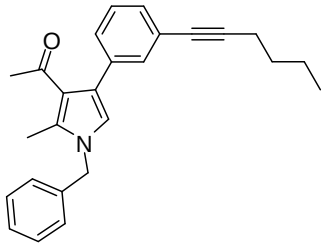
Light brown sticky liquid (307 mg, 86%); IR (neat) 3018, 2927, 2854, 2234, 1686, 1616, 1532, 1497, 1383, 1326, 1286, 1116, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.43 (s, 3H), 2.71 (t, *J* = 6.4 Hz, 2H), 3.83 (t, *J* = 6.4 Hz, 2H), 5.05(s, 2H), 6.53 (s, 1H), 7.08 (d, *J* = 6.8 Hz, 2H), 7.23-7.40 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ11.5, 23.7, 31.0, 36.5, 50.2, 61.0, 82.2, 86.5, 120.2, 121.9, 123.2, 125.0, 126.6, 127.8, 128.1, 129.1, 132.3, 135.2, 197.4; HRMS: *m/z* calcd for $C_{24}H_{24}FNO_2$ (M + 1) 358.1729; found 358.1813.

1-(1-benzyl-2-methyl-4-(3-(oct-1-yn-1-yl)phenyl)-1H-pyrrol-3-yl)ethanone (11c)



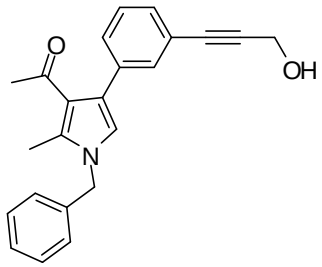
Light brown sticky liquid (322 mg, 81%); IR (neat) 3628, 3062, 2954, 2928, 2857, 2227, 1652, 1599, 1521, 1455, 1353, 1242, 1179, 1078 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, *J*=6.8 Hz, 3H), 1.21-1.46 (m, 6H), 1.56-1.61 (m, 2H), 2.04 (s, 3H), 2.41 (t, *J* = 6.8 Hz, 2H), 2.43 (s, 3H), 5.05(s, 2H), 6.53 (s, 1H), 7.08 (d, *J* = 7.2 Hz, 2H), 7.20-7.38 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ11.5, 14.0, 19.3, 22.5, 28.5, 31.1, 50.2, 80.3, 90.6, 120.1, 121.9, 124.0, 125.1, 126.6, 127.8, 128.0, 129.7, 132.2, 135.2, 136.3, 197.6; HRMS: *m/z* calcd for $C_{28}H_{32}NO$ (M + 1) 398.2406; found 398.2469.

1-(1-benzyl-4-(3-(hex-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (11d)



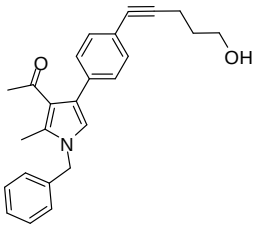
Brown sticky liquid (302 mg, 0.82 mmol, 82%); IR (neat) 2957, 2930, 2871, 2229, 1707, 1652, 1599, 1509, 1454, 1378, 1353, 1240, 1179, 1133, 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.96 (t, *J*=7.2 Hz, 3H), 1.39-1.58 (m, 4H), 2.04 (s, 3H), 2.40 (t, *J* = 6.8 Hz, 2H), 2.43 (s, 3H), 5.05(s, 2H), 6.53 (s, 1H), 7.08 (d, *J* = 6.8 Hz, 2H), 7.20-7.37 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ11.5, 13.6, 19.0, 21.9, 30.7, 31.1, 50.2, 80.3, 90.5, 120.1, 121.9, 123.9, 125.1, 126.6, 127.8, 128.0, 129.7, 132.2, 135.2, 136.3, 197.5; HRMS: *m/z* calcd for $C_{26}H_{28}NO$ (M + 1) 370.2093; found 370.2160.

1-(1-benzyl-4-(3-(3-hydroxyprop-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (11e)



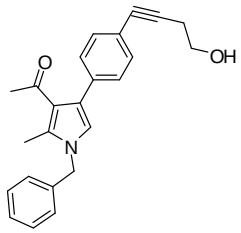
Brown color sticky liquid (274 mg, 80%); IR (neat) 3357, 3063, 3011, 2926, 2855, 2221, 1704, 1645, 1600, 1506, 1480, 1378, 1294, 1216, 1180,1031 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.43 (t, *J*=6.8 Hz, 2H), 2.43 (s, 3H), 4.49 (s, 2H), 5.05(s, 2H), 6.53 (s, 1H), 7.05-7.11 (m, 3H), 7.26-7.42 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 30.9, 50.2, 51.5, 85.2, 87.4, 120.3, 121.7, 122.5, 124.9, 126.6, 127.8, 128.1, 129.5, 132.5, 135.5, 136.3, 147.1, 197.7; HRMS: *m/z* calcd for $C_{23}H_{22}NO_2$ (M + 1) 344.1572; found 344.1649.

1-(1-benzyl-4-(4-(5-hydroxypent-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (12a)



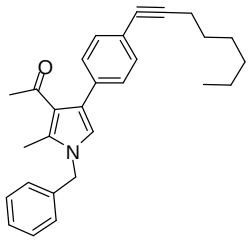
Brown sticky liquid (308 mg, 83%); IR (neat) 3363, 3019, 2929, 2867, 0007, 1647, 1603, 1512, 1419, 1354, 1215, 1154, 1026 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.88-191 (m, 3H), 2.04 (s, 3H), 2.42 (s, 3H), 2.57 (t, *J* = 7.0 Hz, 2H), 3.85 (t, *J* = 7.0 Hz, 2H), 5.05 (s, 2H), 6.53 (s, 1H), 7.09 (d, *J* = 7.0 Hz, 2H), 7.23-7.38 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 16.0, 31.0, 50.3, 61.8, 81.0, 89.6, 120.1, 121.9, 122.0, 125.2, 126.6, 127.8, 128.9, 129.0, 131.4, 135.2, 136.4, 197.5; HRMS: *m/z* calcd for $C_{25}H_{26}NO_2$ (M + 1) 372.1885; found 372.1964.

1-(1-benzyl-4-(4-(4-hydroxybut-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (12b)



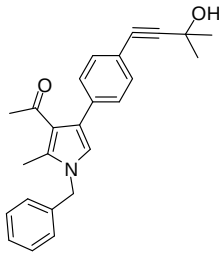
Brown sticky liquid (293 mg, 82%); IR (neat) 3671, 3422, 3017, 2928, 2855, 2226, 1648, 1605, 1559, 1454, 1419, 1354, 1215, 1136, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.04 (s, 3H), 2.42 (s, 3H), 2.72 (t, *J* = 6.0 Hz, 2H), 3.84 (t, *J* = 6.0 Hz, 2H), 5.05 (s, 2H), 6.54 (s, 1H), 7.09 (d, *J*=7.2 Hz, 2H), 7.24-7.42 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 23.8, 29.6, 31.1, 50.3, 61.1, 82.3, 86.6, 120.1, 121.9, 125.2, 126.6, 127.9, 128.0, 129.1, 131.5, 132.2, 135.3, 136.3, 197.5; HRMS: *m/z* calcd for $C_{24}H_{24}NO_2$ (M + 1) 358.1729; found 358.1797.

1-(1-benzyl-2-methyl-4-(4-(oct-1-yn-1-yl)phenyl)-1H-pyrrol-3-yl)ethanone (12c)



Brown sticky liquid (338 mg, 85%); IR (neat) 3676, 3355, 3066, 3014, 2956, 2930, 2858, 2226, 1913, 1648, 1556, 1497, 1419, 1379, 1216, 1179, 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92(d, *J* = 6.8 Hz, 3H), 1.25-1.56 (m, 4H),1.59-1.64 (m, 4H), 2.04 (s, 3H), 2.39-2.41 (m, 2H), 2.42 (s, 3H), 5.05(s, 2H), 6.53 (s, 1H), 7.09 (d, *J* = 7.0 Hz, 2H), 7.22-7.39 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.4, 14.0, 19.4, 22.5, 28.5, 29.6, 31.0, 50.2, 80.3, 90.8, 120.0, 122.0, 125.3, 126.6, 127.6, 128.1, 129.0, 131.3, 135.2, 136.4, 197.5; HRMS: *m/z* calcd for $C_{28}H_{232}NO$ (M + 1) 398.2406; found 398.2477.

1-(1-benzyl-4-(4-(3-hydroxy-3-methylbut-1-yn-1-yl)phenyl)-2-methyl-1H-pyrrol-3-yl)ethanone (12d)



Light yellow solid (297 mg, 80%); mp 84-87 °C; IR (neat) 3671, 3015, 2927, 2225, 1647, 1594, 1509, 1413, 1353, 1294, 1216, 1179, 1079, 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.53 (s, 6H), 2.04 (s, 3H), 2.42 (s, 3H), 5.05 (s, 2H), 6.54 (s, 1H), 7.09 (d, *J* = 7.5 Hz, 2H), 7.25-7.41 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 30.9, 31.4, 50.2, 65.3, 83.9, 94.1, 120.1, 121.1, 125.1, 126.5, 127.8, 128.9, 129.0, 130.1, 131.4, 135.4, 136.2, 197.6; HRMS: *m/z* calcd for $C_{25}H_{26}NO_2$ (M + 1) 372.1885; found 372.1949.

Single crystal X-ray data for compounds 6a and 6h

Single crystals suitable for X-ray diffraction of **6a** and **6h** were grown from methanol. Crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage.

The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Bruker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[1] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[2]

Crystal data of 6a: Molecular formula = $C_{27}H_{25}NO_2$, Formula weight =395.18, Crystal system = Triclinic, space group = $P1$, a = 7.7415 (4) Å, b =10.0575 (5)Å, c = 14.2538 (7)Å, V = 1039.49 (9)Å^3, T = 296 K, Z = 2, D_c = 1.264 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1},16956 reflections measured, 4505 independent reflections, 4128 observed reflections [I > 2.0 σ (I)], R_1_obs = 0.029, Goodness of fit =0.875. Crystallographic data (excluding structure factors) for **6a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 822229.

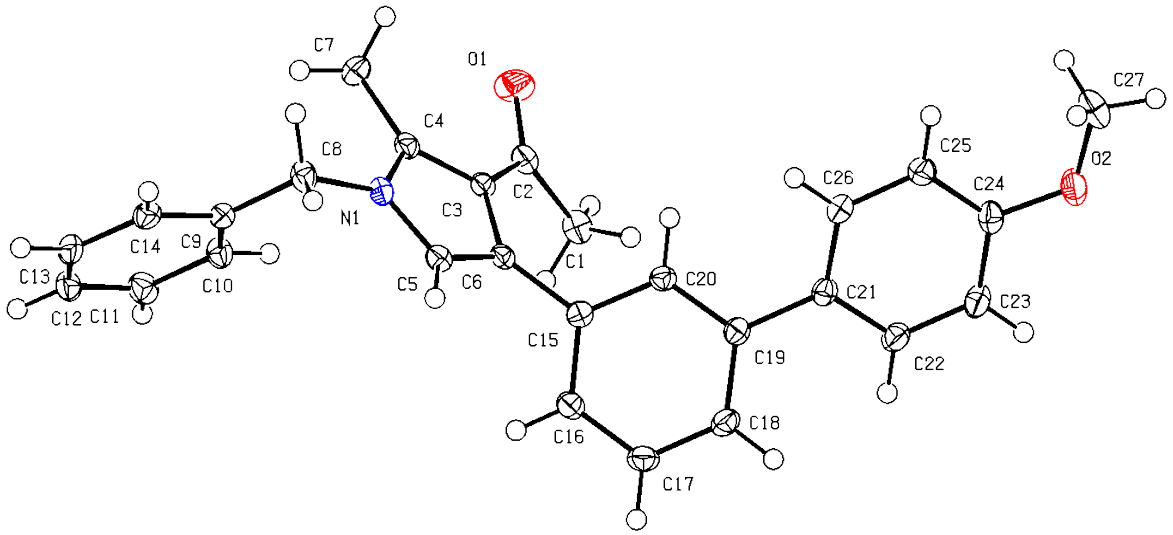


Fig. 1. X-ray crystal structure of **6a** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Crystal data of 6h: Molecular formula = $C_{27}H_{22}F_3NO$, Formula weight = 433.46, Crystal system = Triclinic, space group = *P*1, a = 7.7605 (5) Å, b = 10.2660 (8)Å, c = 14.6746 (11)Å, V = 1054.73 (13)Å^3, T = 296 K, Z = 2, D_c = 0.10 Mg m^{-3}, μ(Mo-Kα) = 0.71073 mm^{-1}, 16632 reflections measured, 4592 independent reflections, 4091 observed reflections [I > 2.0 σ (I)], R_1_obs = 0.037, Goodness of fit =0.803. Crystallographic data (excluding structure factors) for **6h** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 822101.

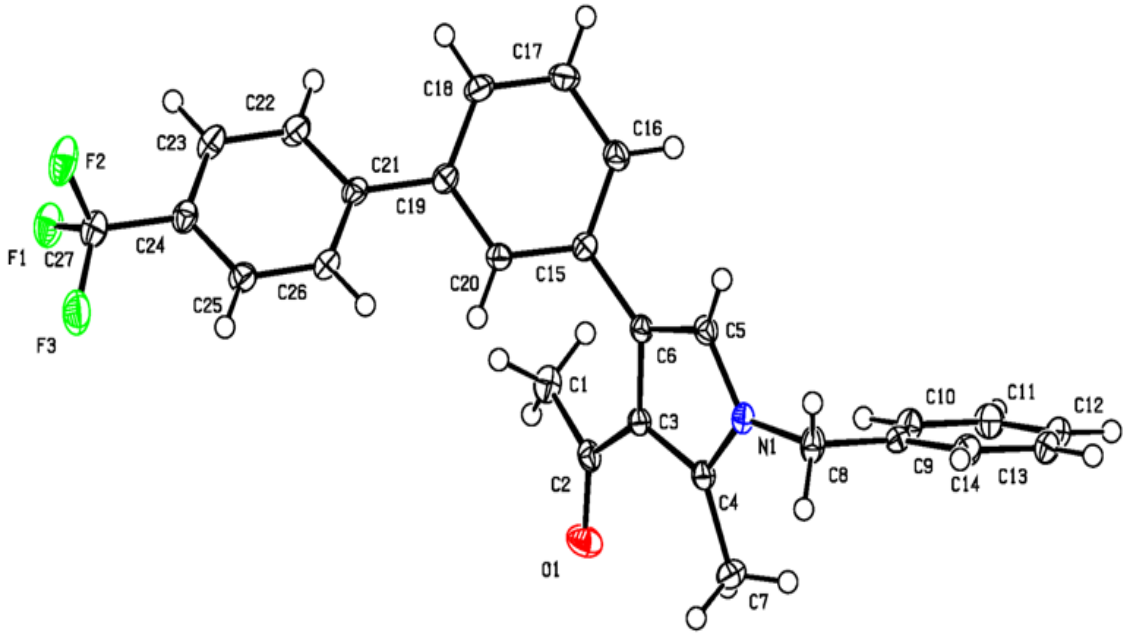


Fig. 2. X-ray crystal structure of **6h** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Reference

1. Bruker SADABS V2008-1, Bruker AXS.: Madison, WI, USA, **2008**.
2. G. M. Sheldrick, SHELX-97, Program for Crystal Structure Determination, University of Göttingen, **1997**.

Pharmacology

Materials and Methods

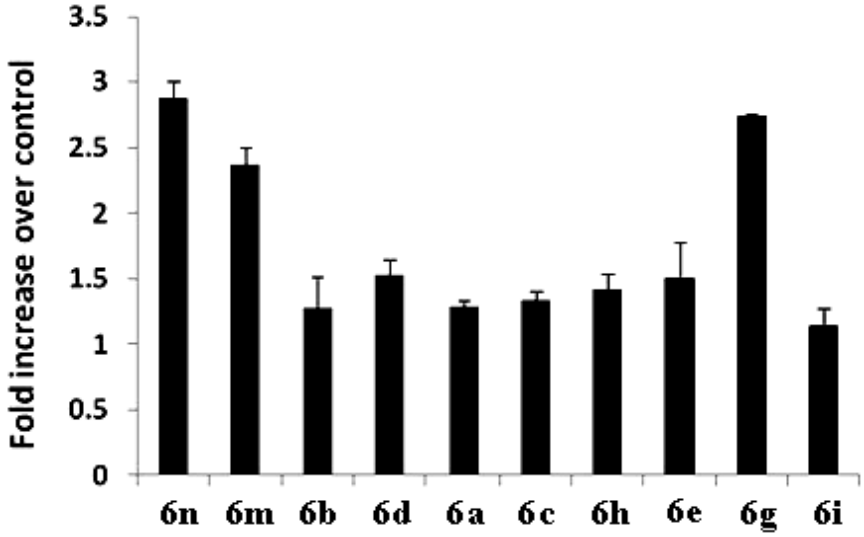
Cells and Reagents

HEK 293 and Sf9 cells were obtained from ATCC (Washington D.C., USA). HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace's supplemented medium (Invitrogen) with 10% FBS. cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland).

Evaluation of PDE4 B inhibition by cell based cAMP reporter assay

One day prior to transfection, HEK 293 cells were seeded in p60 cell culture dish (Tarsons Inc.) and were transfected using Lipofectamine 2000, as per the manufacturer's instructions with 1.2 µg of PDE4B expression plasmid and 4.0 µg of pCRELuc plasmid. After 5 h of transfection, medium was aspirated, cells were trypsinized and seeded in 96 well plates at a density of 60,000 cells/well. Plates were incubated overnight in a CO_2 incubator set to 37^{o}C and 5% CO_2. Twenty four hours post transfection, cells were pre-treated with different compound for 30 minutes, followed by stimulation with 5 µM forskolin for 4 h. Subsequently medium was removed and cells were lysed in reporter lysis buffer (Promega Inc.) for 15 min with gentle rocking at RT. Preliminary screening of the compounds was performed at 30 µM and dose response studies were carried out at eight different concentrations (0.01 to 60 µM). Luciferase activity in the lysates was measured by a Multilabel plate reader (Perklin Elmer 1420 Multilabel counter). Fold elevation of cAMP over forskolin control was calculated using the following formula and the EC_{50} values were determined using GraphPad Prism. Value obtained with forskolin control is set to one. Inhibition of PDE4B by rolipram was used as reference.

$$\text{Fold activation (FA)} = \frac{\text{Normalized value of compound treatment}}{\text{Normalized value of forskolin treatment}}$$



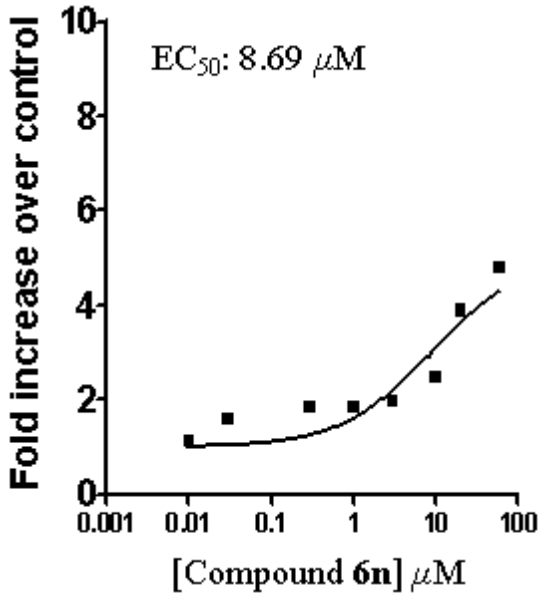


Fig. 3. PDE4B HEK 293 cell based reporter screen of **6** and EC_{50} of **6n**

Reference:

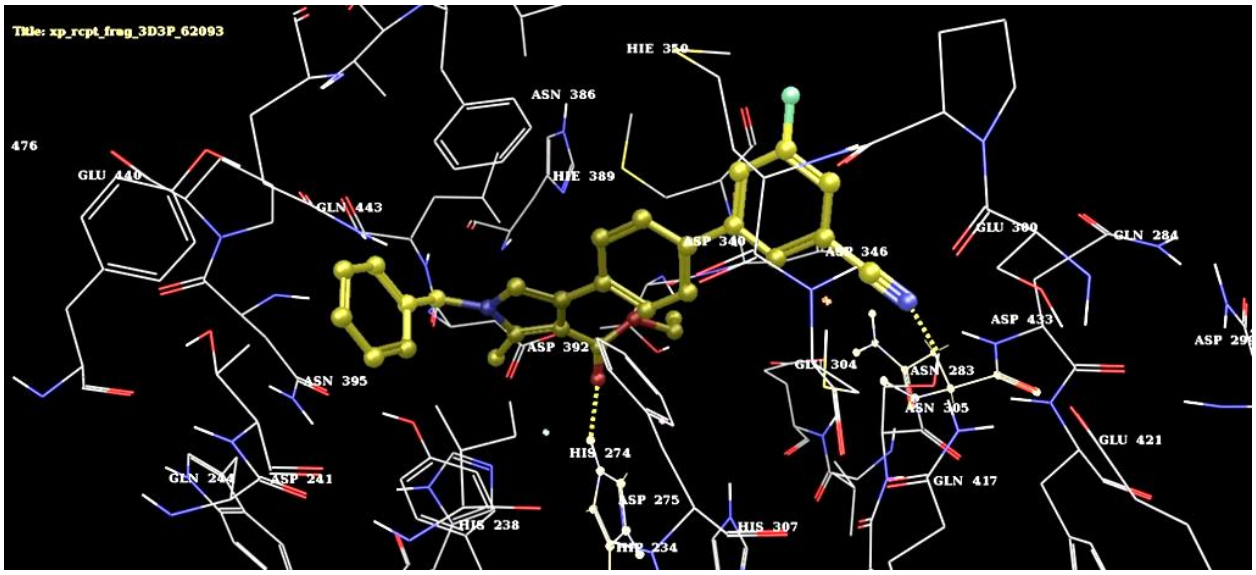
1. P. Wang, J. G.Myers, P. Wu, B. Cheewatrakoolpong, R. W. Egan, M. M. Billah, *Biochem. Biophys. Res. Commun* 1997, **19**, 320-324.

Docking studies

Docking procedure: In the present study we have performed the energy minimization and conformational search with the MACROMODEL application in the Schrodinger package. The molecule **6n** was energy minimized for flexibility followed by the conformational search. We used OPLS_2005 force field and water as implicit solvent. We have followed the PRCG (Polak-Ribier conjugate gradient) method of minimization with 500 iterations with a threshold gradient on 0.05kJ/mol. The conformational search was based on Montecarlo multiple minimum torsional sampling. The ligand molecules were then finally prepared with LIGPREP application.

The PDE4B protein (3D3P) crystal structures was retrieved from the protein data bank and was refined with the PROTEIN PREPERATION WIZARD application in which the hydrogen atoms were added and missing side chains and loops were filled with PRIME application. The water molecules were observed within the 5A distance and other water molecules were deleted beyond 5A from het(hetroatom) groups. Finally the protein is then optimized and minimized with impref using OPLS_2005 force filed. GRID based Docking was carried out in the present study.

Docking of compound 6n



GLIDE SCORE = - 10.93 Kcal/mol