
David Genest, Christophe Rochais, Cédric Lecoutey, Jana Sopková-de Oliveira Santos, Céline Ballandonne, Sabrina Butt-Gueulle, Remi Legay, Marc Since, and Patrick Dallemagne

Supporting Information

Experimental part

1. General
All commercial solvents and reagents were used as-received. The microwave reactions were performed using a Biotage Initiator Microwave oven using 2-5 mL sealed vials; temperatures were measured with an IR-sensor and reaction times given as hold times. Flash chromatography was realized on a spot 2 apparatus. Melting points were determined on a Kofler melting point apparatus and were uncorrected. IR spectra were recorded on KBr discs; only selected absorbances were quoted. TLC were carried out on 5x10 pre-coated plates with silica gel GF254 type 60; LC/MS (ESI) analyses were realized with a separating module using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 5 min; this ratio was hold during 2 min before return to initial conditions in 1 min. Initial conditions were then maintained for 2 min (A: H2O, B: CH3CN; each containing HCOOH: 0.1%); Column: C18, flow : 0.4 mL.min−1). MS detection was performed by positive or negative ESI. High Resolution Mass Spectra were performed at 40 eV by electronic impact (HREIMS) or positive or negative electrospray (HRESIMS). 1H and 13C NMR spectra were recorded, respectively, at 400 and 100 MHz using CDCl3, d6-DMSO, CD3OD or (CD3)2CO as solvents. 2D NMR spectra and 1D NOESY experiments were recorded at 500 MHz. The apparent multiplicity is described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), m (multiplet); chemical shifts δ are reported in parts per million with the solvent resonance as the internal standard; coupling constants J are given in Hertz.

2. Chemistry

2.1. N−{(2E)-2-[(Dimethylamino)methylene]-5,6-dimethoxy-3-oxo-2,3-dihydro-1H-inden-1-yl}-2,2,2-trifluoroacetamide (2a)
To a suspension of 10 g of 1a (33 mmol) in toluene (150 mL) was added DMF-DMA (8.8 mL, 2 equiv). The suspension was then heated at reflux for 8 h, then cooled to room temperature and concentrated under reduced pressure. The resulting orange powder obtained was triturated in ice-cold ether and filtered, which led to 2a as a yellow powder (11.1 g, 94%); mp > 260°C; IR (KBr) ν (cm−1) 2999, 2972, 2937, 2839, 1702, 1663, 1589, 1533, 1501, 1472, 1436, 1360, 1310, 1283, 1207, 1184, 1145, 1128, 1097, 1033, 999; 1H NMR (400 MHz, CDCl3) δ 8.26 (d, J = 7.83 Hz, 1H, NH), 7.05 (s, 1H, C_HNMe2), 7.04 (s, 1H, Hphenyl), 7.01 (s, 1H, Hphenyl), 6.25 (1H, d, J = 8.79 Hz, C_HNH), 3.92 (3H, s, OCH3), 3.90 (3H, s, OCH3), 3.06 (6H, s, NMe2); 13C NMR (100 MHz, CDCl3) δ 191.0, 157.8 (q, J = 37.4 Hz, COCF3), 153.9, 150.2, 147.4 (2C), 141.8, 131.3, 116.2 (q, J = 287.8 Hz, COCF3), 106.4, 103.4, 103.0, 56.4, 56.0, 50.0 (2C); HREIMS [M+] m/z 359,1219 (calcd for C16H17F3N2O4 359,1219).

2.2. N−{(5E)-5-[(Dimethylamino)methylene]-6-oxo-5,6-dihydro-4H-cyclopenta[b]thien-4-yl}-2,2,2-trifluoroacetamide (2b)
Starting from 1b (15 g, 60.2 mmol) and DMF-DMA (16 mL, 120 mmol, 2 equiv) following the same procedure as above, 2b was obtained as a yellow powder (17.95 g, 98%); mp > 260°C; IR (KBr) ν (cm−1) 3184, 2972, 2937, 2839, 1702, 1663, 1589, 1533, 1501, 1472, 1436, 1360, 1310, 1283, 1207, 1184, 1145, 1128, 1097, 1033, 999; 1H NMR (400 MHz, CDCl3) δ 9.09 (d, J = 8.03 Hz, 1H, NH), 7.05 (s, 1H, C_HNMe2), 7.04 (s, 1H, Hphenyl), 7.01 (s, 1H, Hphenyl), 6.25 (1H, d, J = 8.79 Hz, C_HNH), 3.92 (3H, s, OCH3), 3.90 (3H, s, OCH3), 3.06 (6H, s, NMe2); 13C NMR (100 MHz, CDCl3) δ 191.0, 157.8 (q, J = 37.4 Hz, COCF3), 153.9, 150.2, 147.4 (2C), 141.8, 131.3, 116.2 (q, J = 287.8 Hz, COCF3), 106.4, 103.4, 103.0, 56.4, 56.0, 50.0 (2C); HREIMS [M+] m/z 359,1219 (calcd for C16H17F3N2O4 359,1219).
2.3. N-{(5E)-1,3-Dibromo-5-[(dimethylamino)methylene]-6-oxo-5,6-dihydro-4H-cyclopenta[c]thien-4-yl]-2,2,2-trifluoroacetamide (2c)
Starting from 1c (10 g, 21.6 mmol) and DMF-DMA (5.8 mL, 43.3 mmol, 2 equiv) following the same procedure as above, 2c was obtained as a yellow powder (10.8 g, 95%); mp > 260°C; IR (KBr) ν (cm⁻¹) 1714, 1666, 1591, 1542, 1491, 1430, 1406, 1375, 1278, 1208, 1185, 1146, 1105; ¹H NMR (400 MHz, d6-DMSO) δ 10.02 (1H, d, J = 8.75 Hz, NH), 7.36 (1H, s, C(2)NMe2), 6.09 (1H, d, J = 8.75 Hz, CHNMe2), 3.10 (s, 6H, NMe2); ¹³C NMR (100 MHz, d6-DMSO) δ 180.8, 155.4 (d, J = 36.6 Hz, COCF₃), 149.7, 147.6, 143.9, 115.8 (d, J = 288.5 Hz, COCF₃), 108.0, 106.0, 104.8, 54.4, 45.5 (2C); HRESIMS [M+H] m/z 460,8778 (caked for C₁₃H₉Br₂F₃N₃O₃ 460,8782).

2.4. N-(6,7-Dimethoxy-2,4-dihydroindeno[1,2-c]pyrazol-4-yl)-2,2,2-trifluoroacetamide (3a)
To a suspension of 5 g of 2a (14 mmol) in AcOH (30 mL) was added hydrazine sulfate (2.18 g, 16.7 mmol, 1.2 equiv). The suspension was then heated at reflux for 4 h, then cooled to room temperature and concentrated under reduced pressure. The crude mixture was then triturated in water, filtered and dried under air. This led to 3a as a yellow powder (4.34 g, 95%); mp > 260°C; IR (KBr) ν (cm⁻¹) 3271, 1702, 1550, 1486, 1384, 1284, 1212, 1127, 1156, 1034; ¹H NMR (400 MHz, d6-DMSO) δ 12.67 (s, 1H, NHpyrazole), 10.00 (d, J = 5.8 Hz, NHCOCF₃), 7.66 (s, 1H, Hpyrazole), 7.18 (s, 1H, Hphenyl), 7.05 (s, 1H, Hphenyl), 5.74 (d, J = 5.8 Hz, 1H, CF₃NH), 3.83 (s, 3H, OCH₃), 3.76 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d6-DMSO) δ 157.6, 157.5 (q, J = 35.7 Hz, COCF₃), 149.8, 148.4, 140.7, 127.2, 124.8, 123.5, 116.1 (q, J = 287.4 Hz, COCF₃), 109.7, 103.4, 55.9 (2C), 48.0; HRESIMS [M+H] m/z 328.0984 (caked for C₁₀H₁₂F₃N₃OS 328,0909).

2.5. N-1,4-Dihydrothieno[3,2'-4,5]cyclopenta[1,2-c]pyrazol-4-yl-2,2,2-trifluoroacetamide (3b)
Starting from 5 g of 2b (16.4 mmol) in AcOH (30 mL) and hydrazine sulfate (2.57 g, 19.7 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO₂, EtOAc/cyclohexane 1/1). This led to 3b as a white powder (4.13 g, 92%); mp > 260°C; IR (KBr) ν (cm⁻¹) 3287, 1697, 1588, 1540, 1364, 1215, 1196, 1175, 1159, 995, 819, 797; ¹H NMR (400 MHz, CD3OD) δ 7.58 (s, 1H, Hpyrazole), 7.47 (d, J = 4.88 Hz, 1H, Hthiophene), 7.12 (d, J = 4.88 Hz, 1H, Hthiophene), 5.78 (s, 1H, CH(NHCOCF₃); ¹³C NMR (100 MHz, d6-DMSO) δ 159.6 (q, J = 37 Hz, COCF₃), 155.6, 154.9, 136.5, 129.9, 128.1, 125.7, 124.0, 117.5 (COCF₃), q, J = 287 Hz), 47.8; HREIMS [M+] m/z 273.0184 (caked for C₁₀H₁₂F₃N₃OS 273,0184).

2.6. N-(5,7-Dibromo-2,4-dihydrothieno[3,4'-4,5]cyclopenta[1,2-c]pyrazol-4-yl)-2,2,2-trifluoroacetamide (3c)
Starting from 5 g of 2c (10.8 mmol) in AcOH (30 mL) and hydrazine sulfate (1.69 g, 13.0 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO₂, gradient from DCM 100% to MeOH/DCM 10/90). This led to 3c as a yellow powder (4.48 g, 96%); mp > 260°C; IR (KBr) ν (cm⁻¹) 3266, 1698, 1579, 1548, 1411, 1366, 1289, 1185, 1042, 935; ¹H NMR (400 MHz, d6-DMSO) δ 13.11 (1H, s, NHPyrazole), 10.09 (1H, d, J = 7.79 Hz, NHCOCF₃), 7.82 (1H, s, Hpyrazole), 5.87 (1H, d, J = 7.79 Hz, CH(NHCOCF₃); ¹³C NMR (100 MHz, d6-DMSO) δ 156.1 (q, J = 36.6 Hz, COCF₃), 150.7 (2C), 139.0, 129.2, 124.9, 115.9 (q, J = 288.3 Hz, COCF₃), 105.8, 97.4, 45.1; HRESIMS [M+H] m/z 428.84086 (caked for C₁₀H₁₂F₃N₃OS 428,83929).

2.7. N-(6,7-Dimethoxy-1-methyl-1,4-dihydroindeno[1,2-c] pyrazol-4-yl)-2,2,2-trifluoroacetamide (4a)
Starting from 5 g of 2a (14 mmol) in AcOH (30 mL) and methyl-hydrazine (0.88 mL, 16.7 mmol, 1.2 equiv) following the same procedure as above, 4a was obtained as a white powder which was pure enough for use in the next step (4.62 g, 97%); mp 229-230°C; IR (KBr) ν (cm⁻¹) 3276, 1700, 1618, 1553, 1489, 1340, 1307, 1267, 1212, 1187, 1148, 1029; ¹H NMR (400 MHz, d6-DMSO) δ 9.98 (s, 1H, NH), 7.33 (s, 1H, Hpyrazole), 7.30 (s, 1H, Hphenyl), 7.08 (s, 1H, Hphenyl), 5.62 (s, 1H, CF₃NH), 4.04 (s, 3H, NCH₃), 3.86 (s, 3H, OCH₃), 3.76 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d6-DMSO) δ 157.3 (q, J = 37.2 Hz, COCF₃), 149.3, 148.2, 147.9, 141.2, 132.8, 125.6, 123.8, 115.9 (q, J = 288.8 Hz, COCF₃), 110.0, 103.6, 56.1, 55.8, 47.5, 37.3; HREIMS [M+] m/z 341.09994 (caked for C₁₃H₁₄F₃N₃O₃ 341,09869).
2.8. 2,2,2-Trifluoro-N-(1-methyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (4b)

Starting from 5 g of 2b (16.4 mmol) in AcOH (30 mL) and methylhydrazine (1.06 mL, 19.7 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO2, EtOAc/cyclohexane 1/1) to give 4b as a white powder (4.57 g, 97%); mp 228°C; IR (KBr) ν (cm⁻¹) 3276, 1700, 1548, 1447, 1365, 1227, 1205, 1188, 1167, 1153, 1007, 727, 717; ¹H NMR (400 MHz, CDCl₃) δ 7.37 (s, 1H, Hpyrazole), 7.31 (d, J = 4.00 Hz, 1H, Hthiophene), 7.16 (d, J = 4.00 Hz, 1H, Hthiophene), 6.85 (d, J = 4.80 Hz, 1H, NH), 5.71 (d, J = 4.80 Hz, 1H, CH₃NHCOCF₃), 3.89 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 158.1 (COCF₃, q, J = 38 Hz), 153.6, 145.3, 134.2, 131.9, 128.1, 127.7, 123.8, 115.8 (COCF₃, q, J = 288 Hz), 46.9, 37.7; HREIMS [M+] m/z 287.0326 (calcd for C₁₁H₁₀F₃N₃OS 287.034).

2.9. N-(5,7-Dibromo-1-methyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)-2,2,2-trifluoroacetamide (4c)

Starting from 5 g of 2c (10.8 mmol) in AcOH (30 mL) and methylhydrazine (0.70 mL, 13 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO₂, MeOH/DCM gradient from 0/100 to 5/95). This led to 4c as a beige powder (4.62 g, 96%); mp 260°C; IR (KBr) ν (cm⁻¹) 3267, 1703, 1559, 1440, 1215, 1185, 1150, 1028, 941, 725; ¹H NMR (400 MHz, CDCl₃) δ 7.42 (s, 1H, Hpyrazole), 6.49 (d, J = 8.80 Hz, 1H, NH), 5.84 (d, J = 8.80 Hz, 1H, CF₃(NH)), 4.22 (s, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 157.1 (q, J = 18.9 Hz, COCF₃), 149.7, 141.8, 134.4, 134.2, 131.7, 115.7 (q, J = 288.0 Hz, COCF₃), 107.7, 98.5, 44.9, 40.1; HREIMS [M+H] m/z 443.8644 (calcd for C₁₅H₉Br₂F₃N₃OS 443.8629).

2.10. N-(6,7-Dimethoxy-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)-2,2,2-trifluoroacetamide (5a)

Starting from 5 g of 2a (14 mmol) in AcOH (30 mL) and phenylhydrazine (1.65 mL, 16.7 mmol, 1.2 equiv) following the same procedure as above, 5a was obtained as a white powder which was pure enough for use in the next step (5.52 g, 98%); mp 220°C; IR (KBr) ν (cm⁻¹) 3288, 1697, 1652, 1600, 1534, 1485, 1443, 1347, 1262, 1212, 1184, 1151, 1060, 763, 697; ¹H NMR (400 MHz, d6-DMSO) δ 10.09 (d, J = 7.75 Hz, 1H, NH), 7.72 (m, 2H, Hphenyl), 7.70 (s, 1H, Hpyrazole), 7.65 (m, 2H, Hphenyl), 7.49 (m, 1H, Hphenyl), 7.14 (s, 1H, Hphenyl), 5.76 (d, J = 7.75 Hz, 1H, CF₃(NH)), 3.78 (s, 3H, OCH₃), 3.71 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d6-DMSO) δ 157.4 (q, J = 148 Hz, COCF₃), 148.9, 148.6, 146.9, 142.0, 139.3, 135.3, 129.7 (2C), 128.1, 127.9, 123.4, 122.6 (2C), 115.9 (q, J = 288 Hz, COCF₃), 110.0, 103.4, 55.8, 55.5, 47.5; HREIMS [M+H] m/z 404.1236 (calcd for C₂₀H₁₈F₃N₃O₃ 404.122).

2.11. 2,2,2-Trifluoro-N-(1-phenyl-1,4-dihydrothieno [3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (5b)

Starting from 5.5 g of 2b (16.4 mmol) in AcOH (30 mL) and phenylhydrazine (1.96 mL, 19.7 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO₂, EtOAc/cyclohexane 20/80). This led to 5b as a yellow powder (5.28 g, 92%); mp 212°C; IR (KBr) ν (cm⁻¹) 3069, 1701, 1600, 1548, 1530, 1512, 1441, 1367, 1358, 1207, 1186, 1153, 1056, 1010, 946, 757; ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, J = 8.50 Hz, 2H, Hphenyl), 7.55 (s, 1H, Hpyrazole), 7.50 (t, J = 8.50 Hz, 2H, Hphenyl), 7.36 (t, J = 8.50 Hz, 1H, Hphenyl), 7.27 (d, J = 4.90 Hz, 1H, Hthiophene), 7.13 (d, J = 4.90 Hz, 1H, Hthiophene), 7.09 (d, J = 7.90 Hz, 1H, NH), 5.73 (d, J = 7.90 Hz, 1H, CH₃NHCOCF₃); ¹³C NMR (100 MHz, CDCl₃) δ 158.0 (q, J = 37.7 Hz, COCF₃), 153.9, 143.6, 139.2, 135.6, 132.5, 129.7 (2C), 129.4, 127.9, 127.4, 123.7, 120.1 (2C), 115.7 (q, J = 287.9 Hz, COCF₃), 46.5; HREIMS [M+] m/z 349.0497 (calcd for C₁₅H₁₀F₃N₃OS 349.0497).

2.12. N-(5,7-Dibromo-1-phenyl-1,4-dihydrothieno[3',4':5,4]cyclopenta[1,2-c]pyrazol-4-yl)-2,2,2-trifluoroacetamide (5c)

Starting from 5 g of 2c (10.8 mmol) in AcOH (30 mL) and phenylhydrazine (1.28 mL, 13 mmol, 1.2 equiv) following the same procedure as above, a crude powder was obtained which was purified by column chromatography (SiO₂, MeOH/DCM gradient from 0/100 to 5/95). This led to 5c as a beige powder (5.21 g, 95%); mp 226°C; IR (KBr) ν (cm⁻¹) 3247, 1701, 1599, 1549, 1521, 1496, 1424, 1374, 1215, 1195, 1163, 1155, 1064, 990, 764, 721, 698; ¹H NMR (400 MHz, CDCl₃) δ 7.66 (s, 1H, Hpyrazole), 7.52 (m, 2H, Hphenyl), 7.48 (m, 3H, Hphenyl), 6.55 (d,
191.0522 (calcd for C9H9N3S 191.0517).

Starting from (2 g, 4.64 mmol) and following the same procedure as above, 6b was obtained as a white powder (1.76 g, 96%); mp > 260°C; IR (KBr ν (cm⁻¹) 2915, 2959, 2854, 1585, 1505, 1461, 1441, 1280, 1221, 1129, 1058, 1027; ¹H NMR (400 MHz, d₆-DMSO) δ 8.90 (d, J = 5.2 Hz, 3H, NH₃Cl), 7.70 (s, 1H, Hpyrazole), 7.22 (s, 1H, Hphenyl), 5.07 (q, J = 5.2 Hz, 1H, CH₂), 3.84 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 101.7, 89.3, 71.0; 1H NMR (400 MHz, d₆-DMSO) δ 8.90 (d, J = 5.2 Hz, 3H, NH₃Cl), 7.70 (s, 1H, Hpyrazole), 5.01 (q, J = 5.16 Hz, 1H, CH₂), 3.84 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 101.5, 89.3, 70.9.

150.6, 150.0, 148.2, 147.4, 138.0, 127.2, 126.3, 121.3, 110.9, 103.5, 55.9, 47.3, 45.2; HREIMS [M+H⁺] m/z 232.1085 (calcd for C₁₂H₁₃N₃O₂ 232.1086).

15.7-Dibromo-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-amine dihydrochloride (6c)
Starting from 3c (2 g, 4.64 mmol) and following the same procedure as above, 6c was obtained as a yellow powder (1.51 g, 80%); mp > 260°C; IR (KBr ν (cm⁻¹) 3492, 3183, 2948, 1636, 1575, 1542, 1533, 1428, 1049, 1018, 808, 712, 609; ¹H NMR (400 MHz, d₆-DMSO) δ 13.22 (1H, s, NH₃Cl), 8.31 (3H, s, NH₃Cl), 7.79 (s, 1H, Hpyrazole), 5.26 (s, 1H, CH₂); ¹³C NMR (100 MHz, d₆-DMSO) δ 151.7, 148.6, 139.0, 127.3, 126.8, 107.8, 98.4, 44.7; HREIMS [M+H⁺] m/z 332.8569 (calcd for C₁₃H₁₄Br₂N₃O₂ 332.8570).

16.7-Dimethoxy-1-methyl-1,4-dihydrothieno[1,2-c]pyrazol-4-amine dihydrochloride (7a)
Starting from 4a (2 g, 5.86 mmol) and following the same procedure as above, 7a was obtained as a yellow powder (1.68 g, 90%); mp > 260°C; IR (KBr ν (cm⁻¹) 3541, 3401, 2912, 2862, 2622, 1501, 1310, 1231, 1212, 1135, 1024; ¹H NMR (400 MHz, d₆-DMSO) δ 8.90 (d, J = 4.9 Hz, 3H, NH₃Cl), 7.74 (s, 1H, Hpyrazole), 7.44 (s, 1H, Hphenyl), 7.33 (s, 1H, Hphenyl), 4.95 (q, J = 4.9 Hz, 1H, CH₂N₃Cl), 4.05 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 149.7, 148.3, 148.1, 138.3, 133.8, 124.0, 123.4, 111.4, 103.8, 56.2, 55.9, 46.8, 37.5; HREIMS [M+H⁺] m/z 245.11581 (calcd for C₁₃H₁₂N₃O₂ 245.11641).

17.1-Methyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-amine (7b)
Starting from 4b (2 g, 6.96 mmol) and following the same procedure as above, 7b was obtained as a white powder (1.60 g, 87%); mp > 260°C; IR (KBr ν (cm⁻¹) 2133, 1613, 1587, 1529, 1455, 1360, 1285, 1004, 926, 750, 708; ¹H NMR (400 MHz, d₆-DMSO) δ 9.06 (d, J = 5.36 Hz, 3H, CH₂N₃Cl), 7.63 (d, J = 4.88 Hz, 1H, Hthiophene), 7.47 (s, 1H, Hpyrazole), 7.46 (d, J = 4.88 Hz, 1H, Hthiophene), 4.94 (d, J = 5.36 Hz, 1H, CH₂N₃Cl), 3.91 (s, 3H, CH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 151.6, 144.5, 134.4, 131.8, 129.0, 126.2, 124.6, 45.1, 37.4; HREIMS [M+H⁺] m/z 191.0522 (calcd for C₉H₁₀N₂ 191.0517).

18.5-Dibromo-1-methyl-1,4-dihydrothieno[3',4':5]cyclopenta[1,2-c]pyrazol-4-amine dihydrochloride (7c)
Starting from 4c (2 g, 4.49 mmol) and following the same procedure as above, 7c was obtained as a beige powder (1.59 g, 84%); mp > 260°C; IR (KBr ν (cm⁻¹) 3435, 2861, 2703, 2612, 1592, 1559, 1508, 1444, 1361, 1174, 1078, 1017, 893, 710; ¹H NMR (400 MHz, d₆-DMSO) δ 9.03 (s, 3H, NH₃Cl), 7.69 (s, 1H, Hpyrazole), 5.23 (s, 1H, NH₃Cl).
CH₃NH₄Cl), 4.22 (s, 3H, CH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 148.7, 141.1, 135.4, 134.1, 130.1, 108.2, 98.5, 43.7, 39.8; HREIMS [M⁺]/m/z 346.8727 (calcd for C₇H₅Br₂N₃S 346.8728).

2.19. 6,7-Dimethoxy-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-amine dihydrochloride (8a)

Starting from 5a (2 g, 4.96 mmol) and following the same procedure as above, 8a was obtained as a yellow powder (1.66 g, 88%); mp 254-255°C; IR (KBr) ν (cm⁻¹) 2921, 2600, 1596, 1491, 1455, 1317, 1266, 1216, 1114, 1058, 1082; ¹H NMR (400 MHz, d₆-DMSO) δ 9.06 (d, J = 4.9 Hz, 3H, NH₃⁺Cl⁻), 7.83 (s, 1H, Hpyrazole), 7.79 (s, 1H, Hphenyl), 7.67 (m, 4H, Hphenyl), 7.51 (m, 1H, Hphenyl), 6.96 (s, 1H, Hphenyl), 5.10 (d, J = 4.9 Hz, 1H, CH₃NH₄Cl⁻), 3.81 (s, 3H, OCH₃), 3.72 (s, 3H, OCH₃); ¹³C NMR (100 MHz, d₆-DMSO) δ 149.2, 148.5, 147.4, 139.3, 139.1, 136.3, 129.9 (2C), 128.2, 125.9, 123.5, 122.7 (2C), 111.4, 103.5, 55.9, 55.6, 46.7; HREIMS [M⁺]/m/z 307.1335 (calcd for C₁₉H₁₃N₃O₂ 307.1326).

2.20. 1-Phenyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-amine dihydrochloride (8b)

Starting from 5b (2 g, 5.73 mmol) and following the same procedure as above, 8b was obtained as a yellow powder (1.62 g, 87%); mp ≥ 260°C; IR (KBr) ν (cm⁻¹) 2591, 2707, 2604, 1599, 1526, 1508, 1497, 1451, 1440, 1358, 1050, 986, 753; ¹H NMR (400 MHz, d₆-DMSO) δ 8.78 (s, 3H, NH₃⁺), 7.79 (s, 1H, Hpyrazole), 7.73 (m, 2H), 7.69 (m, 1H), 7.65 (t, J = 7.70 Hz, 2H), 7.46 (m, 2H), 5.13 (s, 1H, CH₃NH₄Cl⁻); ¹³C NMR (100 MHz, d₆-DMSO) δ 152.4, 143.1, 138.9, 136.6, 132.3, 130.2 (2C), 129.2, 128.7, 127.7, 124.6, 119.9 (2C), 45.3; HREIMS [M⁺]/m/z 253.0674 (calcd for C₁₉H₁₃N₃O₂ 253.0674).

2.21. 5,7-Dibromo-1-phenyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-amine dihydrochloride (8c)

Starting from 5c (2 g, 3.94 mmol) and following the same procedure as above, 8c was obtained as a yellow powder (1.62 g, 85%); mp ≥ 215°C; IR (KBr) ν (cm⁻¹) 3434, 2880, 1623, 1599, 1553, 1497, 763, 694; ¹H NMR (400 MHz, d₆-DMSO) δ 9.12 (s, 3H, NH₃⁺), 7.95 (s, 1H, Hpyrazole), 7.58 (m, 5H, Hphenyl), 5.31 (s, 1H, CH₃NH₄Cl⁻); ¹³C NMR (100 MHz, d₆-DMSO) δ 148.8, 141.4, 139.2, 137.2, 133.4, 131.2, 129.4, 129.1 (2C), 126.0 (2C), 108.5, 99.4, 43.7; HREIMS [M⁺]/m/z 408.8874 (calcd for C₁₉H₁₃Br₂N₃S 408.8831).

2.22. 2-Chloro-N-(6,7-dimethoxy-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (9a)

To a suspension of 6a (500 mg, 1.64 mmol) in DCM (10 mL) was added TEA (0.71 mL, 5.26 mmol, 3.2 equiv). After 5 min, the mixture was cooled with an ice bath and a solution of chloroacetyl chloride (144 µL, 1.80 mmol, 1.1 equiv) in DCM (10 mL) was added drop-wise. When the addition was over, the ice bath was removed and the mixture was stirred for 1 h. The solvent was then evaporated under reduced pressure and the crude powder was triturated in water, filtered and dry under air. This led to 9a as a white powder (364 mg, 72%); mp 200-202°C; IR (KBr) ν (cm⁻¹) 2933, 1655, 1592, 1518, 1485, 1465, 1434, 1384, 1281, 1215, 1186, 1173, 1136, 1034; ¹H NMR (400 MHz, CDCl₃) δ 7.50 (s, 1H, Hpyrazole), 7.29 (s, 1H, Hphenyl), 7.08 (s, 1H, Hphenyl), 6.78 (d, J = 7.79 Hz, 1H, NHSO₂Cl⁻), 5.92 (d, J = 7.79 Hz, 1H, CH₃NH₄Cl⁻), 4.19 (d, J = 15.6 Hz, 1H, Ha), 4.14 (d, J = 15.6 Hz, 1H, Hb), 3.96 (s, 3H, OCH₃), 3.93 (s, 3H, OCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 166.7, 159.2, 150.3, 149.5, 141.4, 126.9, 124.8, 124.5, 109.2, 103.8, 56.4, 56.3, 48.6, 42.6; HREIMS [M⁺]/m/z 307.0709 (calcd for C₁₉H₁₄ClN₃O₂ 307.0723).

2.23 2-Chloro-N-(6,1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (9b)

Starting from 6b (500 mg, 2.0 mmol), TEA (0.86 mL, 6.4 mmol, 3.2 equiv), chloroacetyl chloride (175 µL, 2.2 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO₂, gradient from MeOH/DCM 0/100 to 10/90). This led to 9b as a beige powder (360 mg, 71%); mp 173°C; IR (KBr) ν (cm⁻¹) 2924, 1652, 1534, 1225, 1105, 996, 960, 784; ¹H NMR (400 MHz, CDCl₃) δ 7.49 (s, 1H, Hpyrazole), 7.37 (d, J = 4.88 Hz, 1H, 7.13 (d, J = 4.88 Hz, 1H), 6.80 (broad d, J = 7.79 Hz, 1H, NHCO), 5.82 (d, J = 7.79 Hz, 1H, CH₃NH₄Cl⁻), 4.16 (d, J = 15.4 Hz, 1H, Ha), 4.12 (d, J = 15.4 Hz, 1H, Hb), 17C NMR (100 MHz, CDCl₃) δ 166.6, 154.8, 153.8, 135.8, 129.1, 128.1, 124.4, 122.8, 47.1, 42.6; HREIMS [M⁺]/m/z 253.007 (calcd for C₁₀H₁₀ClN₃O₂ 253.0077).
22.24. 2-Chloro-N-(5,7-dibromo-1,4-dihydrothieno[3′,4′:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (9c)
Starting from 6c (500 mg, 1.23 mmol), TEA (0.53 mL, 3.92 mmol, 3.2 equiv), chloroacetyl chloride (107 µL, 1.35 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO2, gradient from MeOH/DCM 0/100 to 10/90). This led to 9c as a beige powder (363 mg, 72%); mp 238°C; IR (KBr) ν (cm−1) 3272, 2919, 2874, 1648, 1575, 1537, 1415, 1286, 1227, 1180, 1048, 1022, 985, 968, 813, 794, 612; 1H NMR (400 MHz, d6-DMSO) δ 8.91 (J, D = 8.50 Hz, 1H, NHCO), 7.71 (s, 1H, Hpyrazole), 5.80 (d, J = 8.50 Hz, 1H, CH2(NH)), 4.08 (s, 2H, COCH2); 13C NMR (100 MHz, d6-DMSO) δ 165.7, 151.7, 138.9, 134.1, 130.6, 125.0, 105.4, 97.1, 45.2, 42.3; HREIMS [M+H] m/z 409.8383 (calcd for C10H7Br2ClN3O3 409.8365).

22.25. 2-Chloro-N-(6,7-dimethoxy-1-methyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (10a)
Starting from 7a (500 mg, 1.57 mmol), TEA (0.68 mL, 5.03 mmol, 3.2 equiv), chloroacetyl chloride (138 µL, 1.73 mmol, 1.1 equiv) and following the same procedure as above, 10a was obtained, without need for column chromatography, as a beige powder (369 mg, 73%); mp 238°C; IR (KBr) ν (cm−1) 3258, 3073, 3010, 2939, 1655, 1551, 1522, 1488, 1341, 1307, 1267, 1222, 1128, 1030; 1H NMR (400 MHz, CDCl3) δ 7.40 (s, 1H, Hpyrazole), 7.13 (s, 1H, Hphenyl), 6.98 (s, 1H, Hphenyl), 6.71 (d, J = 8.75 Hz, 1H, NH), 5.83 (d, J = 8.75 Hz, 1H, CH2(NH)), 4.17 (s, 2H, CH2), 4.09 (s, 3H, NCH3), 3.97 (s, 3H, OCH3), 3.92 (s, 3H, OCH3); 13C NMR (100 MHz, CDCl3) δ 166.8, 149.7, 149.0, 148.7, 142.9, 133.5, 126.4, 124.1, 110.2, 102.7, 56.5, 56.4, 48.2, 42.6, 37.6; HREIMS [M+] m/z 321.0876 (calcd for C15H18ClN3O3 321.0879).

22.26. 2-Chloro-N-(1-methyl-1,4-dihydrothieno[3′,2′:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (10b)
Starting from 7b (500 mg, 1.89 mmol), TEA (0.82 mL, 6.06 mmol, 3.2 equiv), chloroacetyl chloride (166 µL, 2.08 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO2, gradient from MeOH/DCM 0/100 to 5/95). This led to 10b as a white powder (380 mg, 75%); mp 230°C; IR (KBr) ν (cm−1) 3261, 1653, 1545, 1446, 1236, 1010, 717; 1H NMR (400 MHz, CDCl3) δ 7.39 (s, 1H, Hpyrazole), 7.28 (d, J = 4.9 Hz, 1H, Hthiophene), 7.14 (d, J = 4.9 Hz, 1H, Hthiophene), 6.81 (d, J = 8.0 Hz, 1H, NH), 5.73 (d, J = 8.0 Hz, 1H, CH2(NH)), 4.14 (s, 2H, CH2), 3.96 (s, 3H, CH3), 4.06 (s, 3H, CH3); 13C NMR (100 MHz, CDCl3) δ 166.7, 154.6, 145.1, 134.0, 131.5, 128.7, 127.5, 47.0, 42.5, 37.6; HREIMS [M+] m/z 321.0876 (calcd for C15H18ClN3O3 321.0879).

22.27. 2-Chloro-N-(5,7-dibromo-1-methyl-1,4-dihydrothieno[3′,4′:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (10c)
Starting from 7c (500 mg, 1.18 mmol), TEA (0.51 mL, 3.79 mmol, 3.2 equiv), chloroacetyl chloride (104 µL, 1.30 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO2, gradient from MeOH/DCM 0/100 to 10/90). This led to 10c as a beige powder (373 mg, 74%); mp 236°C; IR (KBr) ν (cm−1) 3431, 1655, 1557, 1440, 1026, 714; 1H NMR (400 MHz, d6-DMSO) δ 8.75 (d, J = 8.40 Hz, 1H, NH), 7.28 (s, 1H, Hpyrazole), 5.58 (d, J = 8.40 Hz, 1H, CH2(NH)), 4.06 (s, 3H, CH3), 4.00 (s, 2H, CH2); 13C NMR (100 MHz, d6-DMSO) δ 165.9, 151.9, 140.6, 134.3, 134.1, 133.6, 105.9, 97.1, 44.5, 42.3, 39.7; HREIMS [M+H] m/z 423.8511 (calcd for C11H8Br2ClN3O3 423.8522).

22.28. 2-Chloro-N-(6,7-dimethoxy-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (11a)
Starting from 8a (500 mg, 1.31 mmol), TEA (0.57 mL, 4.21 mmol, 3.2 equiv), chloroacetyl chloride (115 µL, 1.45 mmol, 1.1 equiv) and following the same procedure as above, 11a was obtained, without need for column chromatography, as a beige powder (358 mg, 71%); mp 216°C; IR (KBr) ν (cm−1) 2939, 1661, 1599, 1531, 1486, 1408, 1350, 1315, 1261, 1216, 1126, 1086, 1060, 1023; 1H NMR (400 MHz, CDCl3) δ 7.71 (m, 2H, Hphenyl), 7.65 (s, 1H, Hpyrazole), 7.57 (m, 2H, Hphenyl), 7.46 (m, 1H), 7.13 (s, 1H, Hphenyl), 6.97 (s, 1H, Hphenyl), 6.81 (d, J = 8.79 Hz, 1H, NH), 5.92 (d, J = 8.79 Hz, 1H, CH2(NH)), 4.20 (s, 2H, CH2), 3.92 (s, 3H, OCH3), 3.81 (s, 3H, OCH3); 13C NMR (100 MHz, CDCl3) δ 166.9, 149.3, 149.1, 147.8, 143.0, 139.8, 135.4, 129.4 (2C), 128.1 (2C), 124.0, 123.2 (2C), 109.7, 103.5, 56.3, 56.2, 48.1, 42.6; HREIMS [M+] m/z 383.10406 (calcd for C20H18ClN3O3 383.10364).
2.29 2-Chloro-N-(1-phenyl-1,4-dihydrothieno[3’,2’:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (11b)

Starting from 8b (500 mg, 1.53 mmol), TEA (0.66 mL, 4.90 mmol, 3.2 equiv), chloroacetyl chloride (134 µL, 1.69 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO2, gradient from MeOH/DCM 0/100 to 10/90). This led to 11b as a white powder (374 mg, 75%); mp 201°C; IR (KBr) ν (cm⁻¹) 2924, 2834, 1662, 1581, 1445, 1380, 1288, 1288, 1134, 1031; 1H NMR (400 MHz, CDCl₃) δ 7.77 (d, J = 8.2 Hz, 2H, Hphenyl), 7.65 (s, 1H, Hpyrazole), 7.56 (t, J = 8.2 Hz, 2H, Hphenyl), 7.40 (t, J = 8.2 Hz, 1H, Hphenyl), 7.29 (d, J = 4.9 Hz, 1H, Hthiophene), 7.19 (d, J = 4.9 Hz, 1H, Hthiophene), 6.82 (d, J = 7.8 Hz, 1H, NH), 5.83 (d, J = 7.8 Hz, C/HNH), 4.18 (s, 2H, CH₂); 13C NMR (100 MHz, CDCl₃) δ 166.7, 155.0, 143.5, 139.5, 135.6, 132.4, 130.8, 129.8 (2C), 127.4, 127.3, 123.9, 120.3 (2C), 46.8, 42.5; HRESIMS [M+H] m/z 330.0474 (calcd for C₁₆H₁₂ClN₃OS 330.0468).

2.30. 2-Chloro-N-(5,7-dibromo-1-phenyl-1,4-dihydrothieno[3’,2’:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (11c)

Starting from 8c (500 mg, 1.03 mmol), TEA (0.45 mL, 3.31 mmol, 3.2 equiv), chloroacetyl chloride (134 µL, 1.14 mmol, 1.1 equiv) and following the same procedure as above, a crude powder was obtained, which was further purified by column chromatography (SiO₂, gradient from MeOH/DCM 0/100 to 10/90). This led to 11c as a beige powder (378 mg, 75%); mp 236°C; IR (KBr) ν (cm⁻¹) 2929, 2834, 1662, 1581, 1492, 1422, 1298, 1258, 1130, 1063, 1021, 996, 917, 832, 762, 696; 1H NMR (400 MHz, CDCl₃) δ 7.71 (s, 1H, Hpyrazole), 7.52 (d, J = 4.9 Hz, 1H, Hphenyl), 7.19 (d, J = 4.9 Hz, 1H, Hthiophene), 6.82 (d, J = 7.8 Hz, 1H, NH), 5.71 (d, J = 4.9 Hz, 1H, Hthiophene), 6.82 (d, J = 7.8 Hz, 1H, NH); 13C NMR (100 MHz, CDCl₃) δ 166.0, 152.1, 140.8, 139.4, 135.6, 135.2, 133.6, 129.1, 129.0(5) (2C), 126.0 (2C), 106.2, 98.0, 44.5, 42.3; HRESIMS [M+H] m/z 485.8661 (calcd for C₂₅H₂₉N₅O₃ 485.8678).

2.31. 2-(4-Benzylpiperazin-1-yl)-N-(6,7-dimethoxy-1,4-dihydroindenol[1,2-c]pyrazol-4-yl)acetamide (12a)

Chloroacetamide 9a (100 mg, 0.32 mmol), benzylpiperazine dihydrochloride (89 mg, 0.36 mmol, 1.1 equiv) and K₂CO₃ (144 mg, 1.04 mmol, 3.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al₂O₃, gradient from MeOH/DCM 0/100 to 10/90). This led to 14a as a yellow powder (51 mg, 35%); mp 152°C; IR (KBr) ν (cm⁻¹) 2929, 2834, 1662, 1581, 1492, 1445, 1380, 1288, 1223, 1134, 1031; 1H NMR (400 MHz, CDCl₃) δ 7.45 (s, 1H, Hpyrazole), 7.27 (m, 6H, Hphenyl), 7.04 (s, 1H, Hphenyl), 5.96 (d, J = 7.79 Hz, 1H, C/HNH), 3.94 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 3.44 (s, 2H, CH₂phenyl), 3.15 (d, J = 16.6 Hz, 1H, Ha), 3.10 (d, J = 16.6 Hz, 1H, Hb), 2.54 (broad m, 4H, Hipiperazine), 2.38 (broad m, 4H, Hipiperazine); 13C NMR (100 MHz, CDCl₃) δ 171.0, 154.6, 150.9, 142.4, 137.6, 133.6, 133.6, 129.1, 129.0(5) (2C), 126.0 (2C), 106.2, 98.0, 44.5, 42.3; HRESIMS [M+H] m/z 448.2339 (calcd for C₂₅H₂₉N₅O₃ 448.2349).

2.32. 2-(4-Benzylpiperazin-1-yl)-N-(1,4-dihydrothieno[3’,2’:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (12b)

Chloroacetamide 9b (100 mg, 0.39 mmol), benzylpiperazine dihydrochloride (108 mg, 0.43 mmol, 1.1 equiv) and K₂CO₃ (174 mg, 1.26 mmol, 3.2 equiv) were introduced in a microwave reactor. Acetone (2.1 mL) was added, the reactor was sealed and irradiated under microwave to a temperature of 170°C for 1 h 30. After cooling to ambient temperature, the same amounts of benzylpiperazine dihydrochloride and K₂CO₃ were added and the reactor was again irradiated at 170°C for 1 h 30. After cooling to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al₂O₃, gradient from MeOH/DCM 0/100 to 10/90). This led to 14b as a yellow powder (43 mg, 28%); mp 146°C; IR (KBr) ν (cm⁻¹) 2933, 2818, 1656, 1501, 1456, 1355, 1297, 1157, 1134, 1012, 911, 736, 700; 1H NMR (400 MHz, CDCl₃) δ 7.44 (s, 1H, Hpyrazole), 7.25 (m, 7H, Hthiophene + 5Hphenyl + NH), 7.02 (d, J = 8.0 Hz, 1H, Hthiophene), 5.71 (d, J = 8.35 Hz, 1H, C/HNH), 3.41 (s, 2H, CH₂CO), 3.12 (s, 2H, CH₂phenyl), 2.50 (broad m, 4H, Hipiperazine), 2.35 (m, 4H, Hipiperazine); 13C NMR (100 MHz, CDCl₃) δ 171.0, 154.6, 150.9, 137.6, 135.4, 129.3, 129.2 (2C), 128.7, 128.3.
(2C), 127.3 (2C), 122.7, 62.8, 61.5, 53.4 (2C), 52.8 (2C), 46.2; HRESIMS [M+H] m/z 394.1691 (calcd for C_{21}H_{23}N_{5}OS 394.1702).

2.33.  \textit{N-}-(5,7-Dibromo-1,4-dihydrothieno[3',4':4,5]cyclopenta[1,2-c]pyrazol-4-yl)-2-(4-benzylpiperazine) acetamide (12c)

Chloroacetamide 9c (100 mg, 0.24 mmol), benzylpiperazine dihydrochloride (66.6 mg, 0.27 mmol, 1.1 equiv) and K_{2}CO_{3} (107.5 mg, 0.78 mmol, 3.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al_{2}O_{3}, gradient from MeOH/DCM 0/100 to 10/90). This led to 14c as a yellow powder (30.8 mg, 23%); mp 144°C; IR (KBr) \nu (cm^{-1}) 2937, 2816, 1668, 1495, 1454, 1132, 1005, 910, 733, 699; \text{H NMR (400 MHz, CDCl}_{3} \delta 7.42 (s, 1H, Hpyrazole), 7.32 (m, 2H, Hphenyl), 7.28 (m, 2H, Hphenyl), 7.21 (m, 1H, Hphenyl), 5.81 (d, J = 8.75 Hz, 1H, CH_{2}NH), 3.54 (s, 2H, COCH_{2}), 3.29 (s, 2H, CH_{2}phenyl), 2.54 (m, 8H, Hpiperazine); \text{C NMR (100 MHz, CDCl}_{3} \delta 170.8, 150.4, 141.7, 137.2, 134.3, 134.1, 134.0, 128.9 (2C), 128.1 (2C), 127.2, 106.3, 97.4, 63.2, 62.9, 61.1, 53.8, 52.6, 45.7, 44.1; HRESIMS [M+H] m/z 549.9902 (calcd for C_{21}H_{21}Br_{2}N_{5}OS 549.9912).

2.34.  2-(4-Benzylpiperazin-1-yl)-\textit{N-}-(6,7-dimethoxy-1-methyl-1,4-dihydroindenol[1,2-c]pyrazol-4-yl)acetamide (13a)

Chloroacetamide 10a (100 mg, 0.31 mmol), benzylpiperazine dihydrochloride (85.2 mg, 0.34 mmol, 1.1 equiv) and K_{2}CO_{3} (137.4 mg, 0.99 mmol, 3.2 equiv) were refluxed in acetone (2 mL) for 36 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al_{2}O_{3}, gradient from MeOH/DCM 0/100 to 10/90). This led to 15a as a yellow powder (56 mg, 39%); mp 188°C; IR (KBr) \nu (cm^{-1}) 2946, 2819, 1652, 1595, 1484, 1462, 1341, 1309, 1264, 1218, 1130, 1038; \text{H NMR (400 MHz, CDCl}_{3} \delta 7.32 (s, 2H, Hpyrazole + Hphenyl), 7.24 (broad s, 4H, Hphenyl), 7.07 (s, 1H, Hphenyl), 6.94 (s, 1H, Hphenyl), 5.81 (d, J = 8.79 Hz, 1H, CH_{2}NH), 4.03 (s, 3H, NCH_{3}), 3.93 (s, 3H, OCH_{3}), 3.84 (s, 3H, OCH_{3}), 3.40 (s, 2H, CH_{2}phenyl), 3.11 (s, 2H, COCH_{2}), 2.53 (broad m, 4H, Hpiperazine), 2.37 (broad m, 4H, Hpiperazine); \text{C NMR (100 MHz, CDCl}_{3} \delta 170.4, 148.7, 148.0, 147.8, 143.1, 137.3, 132.4, 128.4 (2C), 127.6 (2C), 126.5, 126.4, 123.3, 109.1, 101.9, 62.1, 61.0, 55.7, 55.6, 52.8 (2C), 52.2 (2C), 46.7, 36.9; HRESIMS [M+H] m/z 462.2495 (calcd for C_{26}H_{31}N_{5}O_{3} 462.2505).

2.35.  2-(4-Benzylpiperazine)-\textit{N-}-(1-methyl-1,4-dihydrothieno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (13b)

Chloroacetamide 10b (100 mg, 0.37 mmol), benzylpiperazine dihydrochloride (102.3 mg, 0.41 mmol, 1.1 equiv) and K_{2}CO_{3} (165 mg, 1.20 mmol, 3.2 equiv) were introduced in a microwave reactor. Acetone (2.1 mL) was added and the reactor was sealed and irradiated under microwave to a temperature of 170°C for 1 h 30. After cooling to ambient temperature, the same amounts of benzylpiperazine dihydrochloride and K_{2}CO_{3} were added and the reactor was again irradiated at 170°C for 1 h 30. After cooling to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al_{2}O_{3}, gradient from MeOH/DCM 0/100 to 10/90). This led to 15b as a beige powder (47 mg, 31%); mp 130°C; IR (KBr) \nu (cm^{-1}) 2940, 2821, 1674, 1601, 1488, 1452, 1431, 1155, 1138, 1054, 901, 731, 696; \text{H NMR (400 MHz, CDCl}_{3} \delta 7.35 (s, 1H, Hpyrazole), 7.27 (m, 7H, Hthiophene + 5Hphenyl + NH), 7.10 (d, J = 4.85 Hz, 1H, Hthiophene), 5.78 (d, J = 8.50 Hz, 1H, CH_{2}NH), 3.97 (s, 3H, NCH_{3}), 3.45 (s, 3H, NCH_{3}), 3.45 (s, 2H, CH_{2}CO), 3.09 (s, 2H, CH_{2}phenyl), 2.52 (broad m, 4H, Hphenipiperazine), 2.37 (broad m, 4H, Hphenipiperazine); \text{C NMR (100 MHz, CDCl}_{3} \delta 171.0, 155.7, 145.1, 137.8, 133.7, 131.1, 129.6, 129.1 (2C), 128.3 (2C), 127.3, 127.2, 123.7, 62.8, 61.5, 53.5 (2C), 52.8 (2C), 46.3, 37.6; HRESIMS [M+H] m/z 408.1838 (calcd for C_{22}H_{31}N_{5}O_{3} 408.1858).

2.36.  \textit{N-}-(5,7-Dibromo-1-methyl-1,4-dihydrothieno[3',4':4,5]cyclopenta[1,2-c]pyrazol-4-yl)-2-(4-benzylpiperazine)acetamide (13c)
2.37. 2-(4-Benzylpiperazin-1-yl)-N-(6,7-dimethoxy-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (14a)

Chloroacetamide 10c (100 mg, 0.24 mmol), benzylpiperazine dihydrochloride (64.4 mg, 0.26 mmol, 1.1 equiv) and K2CO3 (104 mg, 0.75 mmol, 3.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al2O3, gradient from MeOH/DCM 0/100 to 10/90) as a yellow powder (45.5 mg, 32%); mp 164°C; IR (KBr) ν (cm⁻¹) 2939, 2815, 1644, 1486, 1350, 1261, 1215, 1134, 1060, 1014, 735, 698; 1H NMR (400 MHz, CDCl3) δ 7.51 (m, 2H, Hphenyl), 7.48 (s, 1H, Hpyrazole), 7.44 (m, 2H, Hphenyl), 7.39 (m, 1H, Hphenyl), 7.29 (m, 2H, Hphenyl), 7.22 (m, 2H, Hphenyl), 7.18 (m, 1H, Hphenyl), 7.09 (s, 1H, Hphenyl), 6.75 (m, 1H, Hphenyl), 6.27 (d, J = 8.40 Hz, 1H, NH), 5.79 (d, J = 8.40 Hz, 1H, CH=NH), 5.81 (s, 3H, OCH3), 3.76 (s, 3H, OCH3), 3.15 (s, 2H, CH2phenyl), 2.92 (s, 2H, CH2phenyl), 2.53 (broad m, 8H, 4*CH2piperazine); 13C NMR (100 MHz, CDCl3) δ 173.7, 149.2, 147.4, 144.1, 140.3, 138.5, 135.1, 129.2 (2C), 129.0 (2C), 128.7, 128.3 (2C), 128.0, 126.7 (2C), 123.2, 122.7 (2C), 110.2, 102.8, 63.0, 58.4, 56.1, 55.8, 53.4 (2C), 53.0 (2C), 47.7; HRESIMS [M+H] m/z 524.2640 (calcd for C31H33N5O3 524.2662).

2.38. 2-(4-Benzylpiperazin-1-yl)-N-(1-phenyl-1,4-dihydroindeno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (14b)

Chloroacetamide 11b (100 mg, 0.30 mmol), benzylpiperazine dihydrochloride (83.1 mg, 0.33 mmol, 1.1 equiv) and K2CO3 (134 mg, 0.97 mmol, 3.2 equiv) were introduced in a microwave reactor. Acetone (2.1 mL) was added, the reactor was sealed and irradiated under microwave to a temperature of 170°C for 1 h 30. After cooling to ambient temperature, the same amounts of benzylpiperazine dihydrochloride and K2CO3 were added and the reactor was again irradiated at 170°C for 1 h 30. After cooling to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. This led to 11b as a beige powder (36 mg, 27%); mp 164°C; IR (KBr) ν (cm⁻¹) 2937, 2821, 1644, 1486, 1350, 1261, 1215, 1134, 1060, 1014, 735, 698; 1H NMR (400 MHz, CDCl3) δ 7.51 (m, 2H, Hphenyl), 7.48 (s, 1H, Hpyrazole), 7.44 (m, 2H, Hphenyl), 7.39 (m, 1H, Hphenyl), 7.29 (m, 2H, Hphenyl), 7.22 (m, 2H, Hphenyl), 7.18 (m, 1H, Hphenyl), 7.09 (s, 1H, Hphenyl), 6.75 (m, 1H, Hphenyl), 6.27 (d, J = 8.40 Hz, 1H, NH), 5.79 (d, J = 8.40 Hz, 1H, CH=NH), 5.81 (s, 3H, OCH3), 3.76 (s, 3H, OCH3), 3.15 (s, 2H, CH2phenyl), 2.92 (s, 2H, CH2phenyl), 2.53 (broad m, 8H, 4*CH2piperazine); 13C NMR (100 MHz, CDCl3) δ 173.7, 149.2, 147.4, 144.1, 140.3, 138.5, 135.1, 129.2 (2C), 129.0 (2C), 128.7, 128.3 (2C), 128.0, 126.7 (2C), 123.2, 122.7 (2C), 110.2, 102.8, 63.0, 58.4, 56.1, 55.8, 53.4 (2C), 53.0 (2C), 47.7; HRESIMS [M+H] m/z 524.2640 (calcd for C31H33N5O3 524.2662).

2.39. N-(5,7-Dibromo-1-phenyl-1,4-dihydrothieno[3',4':5]cyclopenta[1,2-c]pyrazol-4-yl)-2-(4-benzylpiperazine)acetamide (14e)

Chloroacetamide 11e (100 mg, 0.21 mmol), benzylpiperazine dihydrochloride (56.2 mg, 0.23 mmol, 1.1 equiv) and K2CO3 (90.7 mg, 0.66 mmol, 3.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. This led to 15c as a yellow powder (36 mg, 27%); mp 152°C; IR (KBr) ν (cm⁻¹) 2939, 2815, 1674, 1496, 1455, 1441, 1157, 1134, 1012, 911, 734, 699; 1H NMR (400 MHz, CDCl3) δ 7.35 (s, 1H, Hpyrazole), 7.31 (m, 2H, Hphenyl), 7.28 (m, 3H, Hphenyl), 5.82 (d, J = 8.95 Hz, 1H, CH=NH), 4.21 (s, 3H, NCH3), 3.49 (s, 2H, CH2phenyl), 3.08 (s, 2H, CH2CO), 2.51 (m, 4H, Hpipperazin), 2.44 (m, 4H, Hpipperazin); 13C NMR (100 MHz, CDCl3) δ 170.4, 151.7, 141.4, 137.8, 134.6, 134.2 (2C), 129.1 (2C), 128.2 (2C), 127.1, 106.2, 79.6, 63.5, 62.8, 61.4, 53.9, 52.8, 45.8, 44.3, 40.0; HRESIMS [M+H]+ m/z 564.0048 (calcd for C23H23Br2N5O5 564.0068).
temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al$_2$O$_3$, gradient from MeOH/DCM 0/100 to 10/90). This led to 16c as a yellow-orange powder (31 mg, 24%); mp 168°C; IR (KBr) ν (cm$^{-1}$) 2817, 1679, 1497, 1455, 1245, 1296, 1157, 1133, 1012, 912, 734, 697; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.52 (m, 2H, Hphenyl), 7.45 (s, 1H, Hpyrazole), 7.40 (m, 2H, Hphenyl), 7.36 (m, 2H, Hphenyl), 7.29 (m, 1H, Hphenyl), 7.24 (m, 2H, Hphenyl), 7.20 (m, 1H, Hphenyl), 5.77 (d, $J$ = 8.80 Hz, 1H, C$_{phenyl}$); 13C NMR (100 MHz, CDCl$_3$) δ 171.0, 161.4 (d, $J$ = 245.8 Hz, C$_{H_{2-fluorophenyl}}$), 154.5, 146.8, 135.4, 131.5, 129.5, 129.0 (2C), 128.1 (2C), 127.3, 127.1, 124.1 (2C), 106.8, 97.3, 62.9, 62.6, 61.1, 54.3, 52.9, 45.5, 44.1; HRESIMS [M+H] m/z 626.0219 (calcd for C$_{27}$H$_{32}$Br$_2$N$_5$OS 626.0225).

2.40  2-[4-(2-Fluorobenzyl)piperazin-1-yl]-N-(6,7-dimethoxy-1,4-dihydroindenophenol-1,2-c|pyrazol-4-yl)acetamide (15a)

Chloroacetamide 9a (100 mg, 0.32 mmol), o-fluorobenzyl-piperazine (59.3 µL, 0.36 mmol, 1.1 equiv) and K$_2$CO$_3$ (53.9 mg, 0.39 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al$_2$O$_3$, gradient from MeOH/DCM 0/100 to 10/90). This led to 17a as a yellow powder (44 mg, 29%); mp 164°C; IR (KBr) ν (cm$^{-1}$) 2936, 2827, 1652, 1589, 1486, 1457, 1383, 1281, 1217, 1136, 1035; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.46 (s, 1H, Hpyrazole), 7.37 (s, 1H, Hphenyl), 7.30 (t, $J$ = 7.60 Hz, 1H, Hfluorophenyl), 7.23 (m, 2H, NH + Hfluorophenyl), 7.05 (m, 1H, Hfluorophenyl), 7.01 (s, 1H, Hfluorophenyl), 6.95 (s, 1H, Hphenyl), 5.78 (d, $J$ = 8.70 Hz, 1H, C$_{phenyl}$); 13C NMR (100 MHz, CDCl$_3$) δ 171.0, 161.1 (d, $J$ = 245.8 Hz, C$_{H_{2-fluorophenyl}}$), 149.3, 148.5, 148.2, 143.2, 133.5, 131.7 (d, $J$ = 4.62 Hz, 128.6 (d, $J$ = 8.12 Hz), 127.1, 124.8 (d, $J$ = 15.1 Hz), 124.2, 123.7 (d, $J$ = 3.45 Hz), 115.1 (d, $J$ = 22.4 Hz), 110.0, 102.2; 61.5, 56.5, 56.2, 55.1 (d, $J$ = 1.60 Hz, C$_{H_2-fluorophenyl}$), 53.4 (2C), 52.2 (2C), 47.1; HRESIMS [M+H] m/z 466.2244 (calcd for C$_{23}$H$_{28}$F$_{2}$N$_5$O$_3$ 466.2254).

2.41  2-[4-(2-Fluorobenzyl)piperazin-1-yl]-N-(1,4-dihydro-thieno[3',2':4,5]cyclopenta[1,2-c|pyrazol-4-yl)acetamide (15b)

Chloroacetamide 9b (100 mg, 0.39 mmol), o-fluorobenzyl-piperazine (72 µL, 0.43 mmol, 1.1 equiv) and K$_2$CO$_3$ (65.4 mg, 0.47 mmol, 1.2 equiv) were introduced in a microwave reactor. Acetone (2 mL) was added, the reactor was sealed and irradiated under microwave to a temperature of 170°C for 1 h 30. After cooling to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al$_2$O$_3$, gradient from MeOH/DCM 0/100 to 10/90). This led to 17b as an orange powder (39 mg, 24%); mp 158°C; IR (KBr) ν (cm$^{-1}$) 2938, 2811, 1664, 1497, 1462, 1365, 1292, 1156, 1134, 1017, 914, 732, 699; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.41 (s, 1H, Hpyrazole), 7.32 (m, 2H, Haromatic + NH), 7.22 (m, 2H, Haromatic), 7.06 (m, 2H, Haromatic), 7.00 (m, 1H, Haromatic), 5.84 (d, $J$ = 8.30 Hz, 1H, C$_{phenyl}$); 13C NMR (100 MHz, CDCl$_3$) δ 171.0, 160.1 (d, $J$ = 246.3 Hz, CF), 154.5, 146.8, 135.4, 131.5, 129.5, 129.0 (d, $J$ = 7.67 Hz), 128.6 (d, $J$ = 28.4 Hz), 125.8, 123.9, 122.6, 121.7, 120.4, 115.3 (d, $J$ = 22.1 Hz), 62.8, 61.5, 55.0, 53.5, 53.4, 52.6, 46.2; HRESIMS [M+H] m/z 412.1594 (calcd for C$_{23}$H$_{28}$F$_{2}$N$_5$O$_3$ 412.1607).

2.42  N-(5,7-Dibromo-1,4-dihydrothieno[3',4':2,5]cyclopenta[1,2-c|pyrazol-4-yl)-2-[4-(2-fluorobenzyl)piperazinacetamide (15c)

Chloroacetamide 9c (100 mg, 0.24 mmol), o-fluorobenzyl-piperazine (44.4 µL, 0.27 mmol, 1.1 equiv) and K$_2$CO$_3$ (40.3 mg, 0.29 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 24 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by
column chromatography (Al2O3, gradient from MeOH/DCM 0/100 to 10/90). This led to 17c as a beige powder (39 mg, 23%); mp 138°C; IR (KBr) ν (cm−1) 2947, 2811, 1673, 1486, 1447, 1125, 1012, 913, 731; 1H NMR (400 MHz, CDCl3) δ 7.38 (s, 1H, Hpyrazole), 7.34 (m, 1H, Hphenyl), 7.28 (m, 1H, Hphenyl), 7.14 (m, 1H, Hphenyl), 7.08 (m, 1H, Hphenyl), 5.79 (broad s, 1H, CH(NH)), 3.63 (s, 2H, COCH3), 3.12 (s, 2H, CH2phenyl), 2.60 (m, 8H, Hpiperezine); 13C NMR (100 MHz, CDCl3) δ 170.2 (CO), 161.1 (d, J = 245.9 Hz, CF), 151.6, 141.2, 134.8, 134.3, 134.2, 131.2 (d, J = 4.36 Hz), 128.9 (d, J = 8.15 Hz), 124.6 (d, J = 14.8 Hz), 124.0 (d, J = 3.47 Hz), 115.1 (d, J = 22.4 Hz), 106.5, 97.4, 61.2, 55.3, 53.4 (2C), 52.5 (2C), 44.1; HRESIMS [M+H] m/z 567.9798 (calcd for C21H21Br2FN5OS 567.9818).

### 2.43. 2-[4-(2-Fluorobenzyl)piperazin-1-yl]-N-(6,7-dimethoxy-1-methyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (16a)

Chloroacetamide 10a (100 mg, 0.26 mmol), o-fluorobenzyl-piperazine (47.6 µL, 0.29 mmol, 1.1 equiv) and K2CO3 (43.2 mg, 0.31 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 36 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al2O3, gradient from MeOH/DCM 0/100 to 10/90). This led to 18a as a beige powder (49 mg, 33%); mp 176°C; IR (KBr) ν (cm−1) 2940, 2825, 1663, 1587, 1489, 1455, 1342, 1305, 1267, 1224, 1135, 1031; 1H NMR (400 MHz, CDCl3) δ 7.35 (d, J = 1.30 Hz, 1H, Hphenyl), 7.32 (t, J = 7.55 Hz, 1H, Hfluorophenyl), 7.23 (m, 2H, NH + Hfluorophenyl), 7.10 (s, 1H, Hpyrazole), 7.08 (m, 1H, Hfluorophenyl), 6.98 (d, J = 1.30 Hz, 1H, Hphenyl), 5.87 (d, J = 8.75 Hz, 1H, C(NH)), 4.09 (s, 3H, NCH3), 3.97 (s, 3H, OCH3), 3.88 (s, 3H, OCH3), 3.52 (s, 2H, CH2-fluorophenyl), 3.12 (s, 2H, CH2CO), 2.54 (broad m, 4H, Hpiperezine), 2.42 (broad m, 4H, Hpiperezine); 13C NMR (100 MHz, CDCl3) δ 171.1, 161.4 (d, J = 246.3 Hz, CF), 149.4, 148.8, 148.5, 143.9, 133.2, 131.5 (d, J = 4.53 Hz), 128.9 (d, J = 8.18 Hz), 127.2, 124.4 (d, J = 14.6 Hz), 124.0, 123.9 (d, J = 3.52 Hz), 115.3 (d, J = 22.3 Hz), 109.9, 102.5, 61.6, 56.4, 56.3, 55.0 (d, J = 1.64 Hz, CH2-fluorophenyl), 53.5 (2C), 52.6 (2C), 47.3, 37.6; HRESIMS [M+H] m/z 480.2406 (calcd for C26H30FN5O3 480.2411).

### 2.44. 2-[4-(2-Fluorobenzyl)piperazin-1-yl]-N-(1-methyl-1,4-dihydroindeno[3',2':4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (16b)

Chloroacetamide 10b (100 mg, 0.37 mmol), o-fluorobenzyl-piperazine (68.2 µL, 0.41 mmol, 1.1 equiv) and K2CO3 (61.9 mg, 0.45 mmol, 1.2 equiv) were introduced in a microwave reactor. Acetone (2.1 mL) was added, the reactor was sealed and irradiated under microwave to a temperature of 170°C for 1 h 30. After cooling to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al2O3, gradient from MeOH/DCM 0/100 to 10/90). This led to 18b as a yellow powder (48 mg, 30%); mp 118°C; IR (KBr) ν (cm−1) 2931, 2807, 1652, 1592, 1495, 1454, 1347, 1221, 1159, 1096, 1062, 1002, 941, 832, 699; 1H NMR (400 MHz, CDCl3) δ 7.34 (m, 2H, Haromatic), 7.28 (m, 1H, Haromatic), 7.22 (m, 2H, Haromatic + NH), 7.09 (m, 2H, Haromatic), 6.99 (m, 1H, Haromatic), 5.77 (d, J = 8.40 Hz, 1H, C(NH)), 3.95 (s, 3H, NCH3), 3.52 (s, 2H, CH2CO), 3.09 (s, 2H, CH2phenyl), 2.52 (s, 4H, Hpiperezine), 2.43 (s, 4H, Hpiperezine); 13C NMR (100 MHz, CDCl3) δ 170.9, 161.3 (d, J = 246.1 Hz, CF), 155.6, 145.0, 133.7, 131.5 (d, J = 4.40 Hz), 131.1, 129.5, 128.9 (d, J = 8.18 Hz), 127.3 (2C), 124.3 (d, J = 14.5 Hz), 123.9 (d, J = 3.52 Hz), 115.2 (d, J = 22.1 Hz), 61.5, 55.0, 53.4 (2C), 52.6 (2C), 46.3, 37.6; HRESIMS [M+H] m/z 426.1756 (calcd for C22H24FN3O3 426.1764).

### 2.45.  N-(5,7-Dibromo-1-methyl-1,4-dihydrothieno[3',4':5]cyclopenta[1,2-c]pyrazol-4-yl)piperazinacetamide (16c)

Chloroacetamide 10c (100 mg, 0.24 mmol), o-fluorobenzyl-piperazine (42.9 µL, 0.26 mmol, 1.1 equiv) and K2CO3 (39 mg, 0.28 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 36 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al2O3, gradient from MeOH/DCM 0/100 to 10/90). This led to 18c as a yellow powder (31.6 mg,
2.46. 2-[4-(2-Fluorobenzyl)piperazin-1-yl]–N-(6,7-dimethoxy-1-phenyl-1,4-dihydroindeno[1,2-c]pyrazol-4-yl)acetamide (17a)

Chloroacetamide 11a (100 mg, 0.26 mmol), o-fluorobenzyl-piperazine (47.6 µL, 0.29 mmol, 1.1 equiv) and K₂CO₃ (43.2 mg, 0.31 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 36 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al₂O₃, gradient from MeOH/DCM 0/100 to 10/90). This led to 19a as a beige powder (48 mg, 34%); mp 152°C; IR (KBr) ν (cm⁻¹) 3434, 2939, 2824, 1659, 1488, 1349, 1261, 1126, 1134, 762; ¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, J = 7.75 Hz, 2H, Hphenyl), 7.60 (s, 1H, Hpyrazole), 7.56 (t, J = 7.75 Hz, 2H, Hphenyl), 7.44 (t, J = 7.75 Hz, 1H, Hphenyl), 7.32 (m, 2H, NH + Hfluorophenyl), 7.21 (m, 1H, Hfluorophenyl), 7.10 (s, 1H, Hphenyl), 7.08 (t, J = 7.95 Hz, 1H, Hfluorophenyl), 6.99 (m, 1H, Hfluorophenyl), 6.97 (s, 1H, Hphenyl), 5.95 (d, J = 8.80 Hz, 1H, C̃F(NH)), 3.88 (s, 2H, OCH₃), 3.81 (s, 3H, OCH₃), 3.53 (s, 2H, CH₂-fluorophenyl), 3.15 (s, 2H, CH₂CO), 2.57 (broad m, 4H, Hpyrazolipiperazine), 2.45 (broad m, 4H, Hpyrazolipiperazine); ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 161.3 (d, J = 171.2 Hz, CO), 161.3 (d, J = 246.3 Hz, CF), 149.1, 149.0, 147.6, 144.1, 139.9, 135.2, 131.5 (d, J = 4.40 Hz, 129.4 (2C), 129.0, 128.9 (d, J = 8.18 Hz), 127.9, 124.4 (d, J = 14.6 Hz), 124.0, 123.9 (d, J = 3.52 Hz), 123.1 (2C), 115.3 (d, J = 22.3 Hz), 109.5, 103.5, 61.6, 56.3, 56.2, 55.0 (d, J = 1.64 Hz, CH₂-fluorophenyl), 53.5 (2C), 52.6 (2C), 47.3; HRESIMS [M+H] m/z 542.2546 (calcd for C₂₃H₂₂BrF₂N₅O₃ 542.2567).

2.47. 2-[4-(2-Fluorobenzyl)piperazin-1-yl]–N-(1-phenyl-1,4-dihydroindeno[3′,2′:4,5]cyclopenta[1,2-c]pyrazol-4-yl)acetamide (17b)

Chloroacetamide 11b (100 mg, 0.30 mmol), o-fluorobenzyl-piperazine (55.4 µL, 0.33 mmol, 1.1 equiv) and K₂CO₃ (50.3 mg, 0.36 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 12 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column chromatography (Al₂O₃, gradient from MeOH/DCM 0/100 to 10/90). This led to 19b as a beige powder (48 mg, 34%); mp 152°C; IR (KBr) ν (cm⁻¹) 3434, 2939, 2824, 1659, 1488, 1349, 1261, 1126, 1134, 762; ¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, J = 7.75 Hz, 2H, Hphenyl), 7.60 (s, 1H, Hpyrazole), 7.56 (t, J = 7.75 Hz, 2H, Hphenyl), 7.44 (t, J = 7.75 Hz, 1H, Hphenyl), 7.32 (m, 2H, NH + Hfluorophenyl), 7.21 (m, 1H, Hfluorophenyl), 7.10 (s, 1H, Hphenyl), 7.08 (t, J = 7.95 Hz, 1H, Hfluorophenyl), 6.99 (m, 1H, Hfluorophenyl), 6.97 (s, 1H, Hphenyl), 5.95 (d, J = 8.80 Hz, 1H, C̃F(NH)), 3.88 (s, 2H, OCH₃), 3.81 (s, 3H, OCH₃), 3.53 (s, 2H, CH₂-fluorophenyl), 3.15 (s, 2H, CH₂CO), 2.57 (broad m, 4H, Hpyrazolipiperazine), 2.45 (broad m, 4H, Hpyrazolipiperazine); ¹³C NMR (100 MHz, CDCl₃) δ 172.0, 161.3 (d, J = 171.2 Hz, CO), 161.3 (d, J = 246.3 Hz, CF), 149.1, 149.0, 147.6, 144.1, 139.9, 135.2, 131.5 (d, J = 4.40 Hz, 129.4 (2C), 129.0, 128.9 (d, J = 8.18 Hz), 127.9, 124.4 (d, J = 14.6 Hz), 124.0, 123.9 (d, J = 3.52 Hz), 123.1 (2C), 115.3 (d, J = 22.3 Hz), 109.5, 103.5, 61.6, 56.3, 56.2, 55.0 (d, J = 1.64 Hz, CH₂-fluorophenyl), 53.5 (2C), 52.6 (2C), 47.3; HRESIMS [M+H] m/z 542.2546 (calcd for C₂₃H₂₂BrF₂N₅O₃ 542.2567).

2.48. N-(5,7-Dibromo-1-phenyl-1,4-dihydroindeno[3′,4′:5]cyclopenta[1,2-c]pyrazol-4-yl)-2-[4-(2-fluorobenzyl)piperazin-1-yl)acetamide (17c)

Chloroacetamide 11c (100 mg, 0.21 mmol), o-fluorobenzyl-piperazine (37.5 µL, 0.23 mmol, 1.1 equiv) and K₂CO₃ (34 mg, 0.25 mmol, 1.2 equiv) were refluxed in acetone (2 mL) for 24 h. After being cooled to ambient temperature, the crude mixture was filtered and the insoluble product was washed with DCM. The filtrates were combined and the solvents were evaporated under reduced pressure. The crude powder obtained was then purified by column...
chromatography (Al₂O₃, gradient from MeOH/DCM 0/100 to 10/90). This led to 19c as a yellow powder (35.7 mg, 27%); mp 162°C; IR (KBr) ν (cm⁻¹) 2943, 2825, 1668, 1489, 1452, 1437, 1233, 1162, 1131, 1015, 907, 765; ¹H NMR (400 MHz, CDCl₃) δ 7.50 (m, 2H, Hphenyl), 7.48 (s, 1H, Hpyrazole), 7.36 (m, 1H, Hphenyl), 7.31 (m, 2H, Hphenyl) : 7.22 (m, 1H, Hphenyl), 7.17 (m, 1H, Hphenyl), 7.05 (m, 1H, Hphenyl), 7.01 (m, 1H, Hphenyl), 6.68 (broad s, 1H, C₃NH), 3.55 (s, 2H, COCH₂), 3.12 (s, 2H, CH₂phenyl), 2.45 (m, 4H, Hpiperazine), 2.38 (m, 4H, Hpiperazine); ¹³C NMR (100 MHz, CDCl₃) δ 170.2 (C O), 161.2 (d, J = 246 Hz, CF), 151.9, 141.2, 140.5, 134.7, 134.5 (2C), 131.2 (d, J = 4.30 Hz), 129.1 (2C), 128.7 (d, J = 8.30 Hz), 126.2, 124.5 (d, J = 14.4 Hz), 124.1 (d, J = 3.63 Hz), 118.9 (2C), 115.3 (d, J = 22.9 Hz), 105.9, 97.6, 61.7, 55.2, 54.1 (2C), 52.5 (2C), 43.8.

3. Biology

3.1. In vitro tests of AChE and BuChE biological activity

Inhibitory capacity of compounds on AChE biological activity was evaluated through the use of the spectrometric method of Ellman. Acetyl- or butyrylthiocholine iodide and 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) were purchased from Sigma Aldrich. Lyophilized electric eel AChE (Type III, Sigma Aldrich), or BuChE from equine serum (Sigma Aldrich) was dissolved in 0.2 M phosphate buffer pH 7.4 such as to have enzyme solutions stock with 2.5 units/ml enzyme activity. AChE from human erythrocytes (buffered aqueous solution, ≥500 units/mg protein (BCA), Sigma Aldrich) was diluted in 20 mM HEPES buffer pH 8, 0.1% Triton X-100 such as to have enzyme solution with 2.5 units/ml enzyme activity. In the procedure, 100 µL of 0.3 mM DTNB dissolved in phosphate buffer pH 7.4 were added into the 96 wells plate followed by 50 µL of test compound solution and 50 µL of enzyme solution. After 5 min of preincubation, the reaction was then initiated by the injection of 50 µL of 10 mM acetyl- or butyrylthiocholine iodide solution. The hydrolysis of acetyl- or butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetyl- or butyrylthiocholine, at a wavelength of 412 nm every minute for 10 min using a 96-well microplate plate reader (TECAN Infinite M200, Lyon, France). Test compounds were dissolved in analytical grade DMSO. Donepezil was used as reference standard.

First screening of AChE and BuChE activity was carried out at a 10⁻⁵ M concentration of compounds under study. For the compounds with significant inhibition (≥ 50%) after 4 min of reaction, IC₅₀ values were determined graphically from 6 points inhibition curves using the Origin software.

3.2. Propidium competition assay

Propidium exhibits an increase in fluorescence on binding to AChE peripheral site, making it a useful probe for competitive ligand binding to the enzyme. Fluorescence was measured in a Tecan Infinite M200 plate reader. Measurements were carried out in 200 µl solution volume, in 96-well plates. The buffer used was 1 mM Tris/HCl, pH 8.0, 5 U eeAChE which was incubated, for 15 min at 25°C, with a 150 µL 10⁻⁵M solution of the compounds or donepezil (from Tocris) as control. One micromolar propidium iodide 50 µL solution was added 10 min before fluorescence measurement. The excitation wavelength was 535 nm, and that of emission, 595 nm. Each assay was repeated, at least, three different times.

3.3. In vitro tests of AChE kinetic studies.

Kinetic studies of compounds on AChE biological activity was evaluated through the use of the spectrometric method of Ellman. Acetylthiocholine iodide, lyophilized electric eel Acetylcholinesterase (Type III, AChE electric eel) and 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) were purchased from Sigma Aldrich. AChE was dissolved in 0.2 M phosphate buffer pH 7.4 such as to have enzyme solutions stock with 0.25 units/ml AChE activity. In the procedure, 100 µL of 0.3 mM DTNB dissolved in phosphate buffer pH 7.4 were added into the 96 wells plate followed by 50 µL of test compound solution and 50 µL of enzyme solution. After 5 min of preincubation, the reaction was then initiated by the addition of 50 µL of different concentrations of acetylthiocholine iodide solution (from 0.02 to 0.2mM). The hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine, at a wavelength of 412 nm every minute for 10 min using a 96-well microplate plate reader (TECAN Infinite M200, Lyon, France). Test compounds were dissolved in analytical grade DMSO.
The kinetic studies were performed using four concentrations of inhibitor (0-1µM).

4. X-Ray Crystallography

Single crystals of 2b and 7a suitable for X-ray crystallographic analysis were obtained by slow evaporation from dichloromethane and water, respectively. Data for crystal structure analysis were collected at 150 K with a Bruker–Nonius Kappa CCD area detector diffractometer with graphite–monochromatised Mo Kα radiation (λ = 0.71073 Å). The structures were solved using direct methods and refined by full-matrix least-squares analysis on F². Crystallographic data: Crystal size: 0.51×0.42×0.09mm. Formula C₁₂H₁₁F₃N₂O₂S, formula weight 304.30, crystal system monoclinic, space group P2₁/c, a = 6.7601(2) Å, b = 8.9249(3) Å, c = 22.5441(5) Å, α = 90°, β = 97.265 (2)°, γ = 90°, V = 1349.24(7) Å³, Z = 4, calculated density = 1.498 g/cm³, μ = 0.277 mm⁻¹, Rint = 0.026, R[F²>2σ(F²)] = 0.047, wR(F²) = 0.112. Crystallographic data of 7a: the compound 7a co-crystalised with two chlorines and two water molecule. Crystal size: 0.40×0.38×0.18mm. Formula C₁₃H₁₇N₃O₂, 2(Cl), 2(H₂O), formula weight 354.23, crystal system triclinic, space group P-1, a = 7.2598(2) Å, b = 10.0853(3) Å, c = 12.2011(3) Å, α = 101.010(1)°, β = 93.643(2)°, γ = 100.4820(10)°, V = 857.77(4) Å³, Z = 2, calculated density = 1.371 g/cm³, μ = 0.398 mm⁻¹, Rint = 0.025, R[F²>2σ(F²)] = 0.042, wR(F²) = 0.121. Software used to solve structures: SHELXS–97. Software used to refine structures: SHELXL–97. Software used to prepare material for publication: SHELXL–97. Crystallographic data for compound 2b CCDC899873 and 7a CCDC899872 have been deposited at the Cambridge Crystallographic Data Centre. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (+44-1223-336408; E-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Figure 1: ORTEP diagram of the crystal majority conformation of 2b (A) and the crystal structure of 7a (B) with the thermal ellipsoid at 50% probability.