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SYNTHESIS AND PHARMACOLOGICAL PROPERTIES OF 1-[(ADAMANTAN-1-YL)-4-PHENYL]-3(4)-PYRAZOLYLUREA AND THIOUREA

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Abstract

A series of 1,3-disubstituted ureas and thioureas containing 1,3,5-trisubstituted pyrazole and (adamantan-1-yl)phenyl moieties were synthesized in 69–89% yields by reacting 1-(4-isocyanatophenyl)adamantane and 1-(4-isothiocyanatophenyl)adamantane with 3- or 4-aminopyrazoles. The inhibitory activity of the obtained compounds against human soluble epoxide hydrolase (sEH) ranged from 15.2 to 51.2 nmol/L, and their solubility in water ranged from 44 to 83 μmol/L.

Keywords: amines, 1,3-disubstituted ureas and thioureas, isocyanates and isothiocyanates, pyrazoles, adamantane derivatives, soluble epoxide hydrolase (sEH), inhibitors.

Introduction

Substituted pyrazoles exhibit a wide range of biological activities, including analgesic and anti-inflammatory [1], antibacterial [2], antifungal [3], antitumor [4], and antiviral [5] activities. They are also being studied as inhibitors of human soluble epoxide hydrolase (sEH) (IC50 220–224 nmol/L) [6]. Data on the inhibitory activity of pyrazole-containing compounds against p38 MAPKα protein kinase, as well as molecular docking results, have shown that the high activity of these compounds is due to the ability of the pyrazole pharmacophore site to bind up to two amino acid residues in the enzyme's active site [7]. 1,3-Disubstituted ureas containing a pyrazole group in their structure are being studied as inhibitors of the cyclin-dependent kinase CDK5 and glycogen synthase kinase GSK3α/β [8], inhibitors of the BRAF sarcoma viral oncogene [9], inhibitors of the tyrosine kinase activity of the VEGFR-2 receptor [10], and selective dual inhibitors of mammalian targets of rapamycin complexes 1 and 2 (mTORC1 and mTORC2) [11]. Inhibition of JNK3 kinase by such structures is a promising strategy in the treatment of neurodegeneration [12]. A number of ureas containing a 1,5-diarylpyrazole group in their structure have been studied as dual inhibitors of cyclooxygenase-2 (COX-2) and sEH [13]. Human sEH is an enzyme involved in the metabolism of epoxy fatty acids (arachidonic acid metabolites) to the corresponding vicinal diols via the catalytic addition of a water molecule [14]. Inhibition of human sEH with target-directed inhibitors has beneficial effects on the treatment of hypertension and kidney diseases [15], as well as inflammatory and pain conditions [16].

Analysis of the sEH gene structure shows that it consists of two globular proteins, each containing its own C-terminal epoxide hydrolase and N-terminal phosphatase domains

for binding to different substrates [17].

Among the existing inhibitors of the C-terminal epoxide hydrolase domain, 1,3-disubstituted ureas occupy an important place. For example, small symmetrical ureas, such as 1,3-dicyclohexylurea, are potent inhibitors of sEH [18]. However, low aqueous solubility severely limits their use as dosage forms. To improve solubility, unsymmetrical ureas with a flexible side chain, such as 12-(1-adamantan-1-ylureido) dodecanoic acid (AUDA), were synthesized. Although this class of sEH inhibitors exhibits high activity in vivo, they are extremely rapidly metabolized, which reduces their applicability [19].

To date, more than 3,000 disubstituted ureas containing aliphatic, aromatic, and heterocyclic moieties have been studied as sEH inhibitors [20]. Many of the synthesized sEH inhibitors are adamantyl-containing ureas with a ureide pharmacophore center. However, compounds combining high activity, aqueous solubility, and metabolic stability have not been found among them.

Materials and Methods

IR spectra were recorded on a Bruker Alpha spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM300 spectrometer (300 and 75 MHz, respectively) in DMSO-d6 and $CDCl_3$ at 25°C, using TMS as an internal standard. ^{13}C NMR signal assignments were based on published data [25, 27]. Mass spectra were recorded on a Finnigan MAT Incos 50 instrument with direct sample injection in IE mode (70 eV). Melting points were determined on a Boetius melting stage (heating rate 4°C/min). Elemental analysis was performed on a PerkinElmer 2400 Series II instrument. The reaction progress and purity of the obtained compounds were monitored by TLC on Merck Silicagel 60 F254 plates, eluent MeCN–$CHCl_3$, 1:5.

The starting materials 1-(isocyanatophenyl)adamantane and 1-(isothiocyanatophenyl)adamantane (2)

[23] and nitropyrazolylacetic acids 5f,g [26] were obtained according to published methods; pyrazoles 3a and 4b–e,h were provided by Crea-Chim.

General procedure for the synthesis of 1,3-disubstituted ureas and thioureas 1a–h.

To a solution of 454 mg (1.0 mmol) of 1-(isocyanatophenyl)adamantane or 1-(isothiocyanatophenyl)adamantane (2) in 5 mL of anhydrous DMF at 0 °C were added 1.0 mmol of aminopyrazole 3a–h and 70 mL (1.0 mmol) of Et_3N. The mixture was stirred at room temperature for 24 h. The precipitated white crystals were filtered, washed with 30 mL of EtOAc, 30 mL of 1 N HCl, and 30 mL of H_2O, and air-dried.

4-[(Adamantan-1-yl)phenyl]-3-[1-benzyl-3,5-dimethyl-1H-pyrazol-4-yl]urea (1a). Yield 381 mg (84%). M.p. 210–212 °C. IR (ν, cm^{-1}): 3318, 2901, 2847, 1643, 1572, 1451, 1301, 1246, 1095, 649. ^{1}H NMR ($CDCl_3$, δ, ppm, J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.31 (3H, t, J = 7.0, H Ph); 7.12 (2H, d, J = 6.9, H Ph); 5.51 (1H, br s, NH); 5.26 (2H, s, NCH_2Ph); 4.51 (1H, br s, NH Ar); 2.24 (3H, s, CH_3); 2.13 (3H, s, CH_3); 1.95 (3H, br s, CH Ad); 1.72–1.54 (6H, m, CH_2 Ad); 1.38 (6H, s, CH_2 Ad). ^{13}C NMR (DMSO-d6, δ, ppm): 157.1(C=O); 143.2; 137.9; 134.6; 128.5 (CH Ph); 127.3(CH Ph); 127.0 (CH Ph); 116.8; 52.3 (CH_2); 51.0 (CH_2); 39.8 (CH_2 Ad); 36.7 (CH_2 Ad); 33.7 (C Ad); 27.8 (CH Ad); 11.2 (CH_3); 9.1 (CH_3). MS (*m/z*, (Irel, %)): 454$[M]^{\bullet+}$ (100). Found, %: C 76.67; H 7.52; N 12.35. $C_{29}H_{34}N_4O$. Calculated, %: C 76.65; H 7.49; N 12.33.

4-[(Adamantan-1-yl)phenyl]-3-[3,5-dimethyl-1-(4-methylbenzyl)-1H-pyrazol-4-yl]urea (1b). Yield 407 mg (87%). M.p. 234–236 °C. IR (ν, cm^{-1}): 3311, 2901, 2845, 1642, 1576, 1473, 1449, 1303, 1248, 1108, 730. ^{1}H NMR ($CDCl_3$, δ, ppm, J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.12 (2H, d, J = 7.7, H Ar); 7.02 (2H, d, J = 7.8, H Ar); 5.46 (1H, s, NH); 5.20 (2H, s, NCH_2Ar); 4.48 (1H, br. s, NH Ar); 2.33 (3H, s, CH_3); 2.23 (3H, s, CH_3); 2.12 (3H, s, CH_3); 1.94 (3H, br. s, CH Ad); 1.72–1.51 (6H, m, CH_2 Ad); 1.37 (6H, s, CH_2 Ad). ^{13}C NMR (DMSO-d6, δ, ppm): 158.2 (C=O); 144.3; 137.6; 135.9; 135.6; 130.2 (CH Ar); 128.1 (CH Ar); 117.8; 53.2 (CH_2); 52.1 (CH_2); 40.9 (CH_2 Ad); 37.8 (CH_2 Ad); 34.9 (C Ad); 28.9 (CH Ad); 21.8 (4-$CH_3C_6H_4$); 12.3 (CH_3); 10.2 (CH_3). MS (*m/z*, (Irel, %): 468 $[M]^{\bullet+}$ (100). Found, %: C 76.94; H 7.71; N 11.95. $C_{30}H_{36}N_4O$. Calculated, %: C 76.92; H 7.69; N 11.97.

4-[(Adamantan-1-yl)phenyl]-3-[3,5-dimethyl-1-(4-chlorobenzyl)-1H-pyrazol-4-yl]urea (1c). Yield 386 mg (79%). M.p. 263–265 °C. IR (ν, cm^{-1}): 3310, 2907, 2845, 1642, 1573, 1490, 1448, 1314, 1249, 1093, 1016, 803, 732. ^{1}H NMR (DMSO-d6, δ, ppm J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.40 (2H, d, J = 8.1, H Ar); 7.16 (2H, d, J = 8.1, H Ar); 7.09 (1H, s, NH); 5.74 (1H, t, J = 5.1, NH Ar); 5.18 (2H, s, NCH_2Ar); 2.02 (3H, s, CH3); 2.01 (3H, s, CH3); 1.93 (3H, br s, CH Ad); 1.70–1.56 (6H, m, CH2 Ad); 1.42 (6H, s, CH_2 Ad). ^{1}H NMR ($CDCl_3$, δ, ppm, J, Hz): 7.30 (2H, d, J = 8.8, H Ar); 7.06(2H, d, J = 8.3, H Ar); 5.50 (1H, s, NH); 5.21 (2H, s, NCH_2Ar); 4.46 (1H, br s, NH Ar); 2.23 (3H, s, CH_3); 2.12 (3H, s, CH_3); 1.95 (3H, br s, CH Ad); 1.73–1.53 (6H, m, CH_2 Ad); 1.38 (6H, s,CH_2 Ad). ^{13}C NMR ($CDCl_3$, δ, ppm): 157.0 (C=O); 143.4; 136.8; 134.4; 131.9; 128.8 (CH Ar); 128.4 (CH Ar); 116.7; 51.4 (CH_2); 50.9 (CH2); 39.7 (CH2 Ad); 36.6 (CH2 Ad); 33.6 (C Ad); 27.8 (CH Ad); 11.1 (CH3); 8.9 (CH_3). MS (*m/z*, (Irel, %): 488 $[M(^{35}Cl)]^{\bullet+}$ (100), 490 $[M(^{37}Cl)]$ (30). Found, %: C 71.35; H 6.80; N 11.50. $C_{29}H_{33}ClN_4O$. Calculated, %: C 71.31; H 6.76; N 11.48.

4-[(Adamantan-1-yl)phenyl]-3-[3,5-dimethyl-1-(4-fluoro-benzyl)-1H-pyrazol-4-yl]urea (1d). Yield 406 mg (86%). M.p. 238–240 °C. IR (ν, cm^{-1}): 3318, 2901, 2847, 1643, 1604, 1573, 1510, 1481, 1449, 1314, 1297,1248, 1230, 1157, 1104, 833, 764, 721, 532. ^{1}H NMR ($CDCl_3$, δ, ppm, J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.12 (2H, d.d, J = 8.4, J = 5.4, H Ar); 7.01 (2H, t, J = 8.5, H Ar); 5.50 (1H, s, NH); 5.21(2H, s, NCH_2Ar); 4.47 (1H, br s, NH Ar); 2.23 (3H, s, CH_3); 2.13 (3H, s, CH_3); 1.94 (3H, br s, CH Ad); 1.72–1.50 (6H, m, CH_2 Ad); 1.38 (6H, s, CH_2 Ad). ^{13}C NMR (DMSO-d6, δ, ppm, J, Hz): 162.6 (d, J = 242.9, C-4' Ar); 158.2 (C=O); 144.5; 135.6; 135.1; 130.3 (d, J = 8.0, C-2',6' Ar); 117.9; 116.4 (d, J = 21.4, C-3',5' Ar); 52.6 (CH_2); 52.1 (CH_2); 40.9(CH_2 Ad); 37.8 (CH_2 Ad); 34.9 (C Ad); 28.9 (CH Ad); 12.3 (CH_3); 10.2 (CH_3). MS (*m/z* (Irel, %): 472 $[M]^{\bullet+}$(100). Found, %: C 73.75; H 6.97; N 11.89. $C_{29}H_{33}FN_4O$. Calculated, %: C 73.73; H 6.99; N 11.86.

4-[(Adamantan-1-yl)phenyl]-3-[3,5-dimethyl-1-(2-chloro-benzyl)-1H-pyrazol-4-yl]urea (1e). Yield 298 mg (61%). M.p. 210–212 °C. IR (ν, cm^{-1}): 3304, 2906, 2842, 1635, 1573, 1488, 1445, 1314, 1248, 1110, 1088, 1018, 830, 725, 696. ^{1}H NMR ($CDCl_3$, δ, ppm, J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.41 (1H, d, J = 7.6, H Ar); 7.25 (2H, d, J = 6.9, H Ar); 6.81 (1H, d, J = 6.9, H Ar); 5.44 (2H, s, NCH_2Ar); 5.40 (1H, s, NH); 5.17 (1H, br. s, NH Ar); 2.29 (3H, s, CH_3); 2.18 (3H, s, CH_3); 1.96 (3H, br. s, CH Ad); 1.73–1.50 (6H, m, CH_2 Ad); 1.44 (6H, s, CH_2 Ad). MS (*m/z* (Irel, %): 488 $[M(^{35}Cl)]^{\bullet+}$ (100), 490 $[M(^{37}Cl)]^{\bullet+}$ (30). Found, %: C 71.36; H 6.74; N 11.43. $C_{29}H_{33}ClN_4O$. Calculated, %: C 71.31; H 6.76; N 11.48.

4-[(Adamantan-1-yl)phenyl]-3-{3,5-dimethyl-1-[2-(morpholin-4-yl)-2-oxoethyl]-1H-pyrazol-4-yl}-urea (1f). Yield 314 mg (64%). IR (ν, cm^{-1}): 3328, 2901, 2847, 1651, 1634, 1571, 1450, 1345, 1311, 1277, 1244, 1117. ^{1}H NMR ($CDCl_3$, δ, ppm J, Hz): 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 5.78 (1H, br. s, NH); 4.93 (2H, s, $NCH_2C(O)$); 4.86 (1H br s, NH Ar); 3.72 (4H, t, J = 4.4, $2OCH_2CH_2N$); 3.59 (4H, t, J = 4.4, $2OCH_2CH_2N$); 2.89 (3H, s, CH_3); 2.21 (3H, s, CH_3); 1.95 (3H, br s, CH Ad); 1.76–1.60 (6H, m, CH_2 Ad); 1.50 (6H, s, CH_2 Ad). MS (*m/z* (Irel, %): 491 $[M]^{\bullet+}$ (100). Found, %: C 68.42; H 7.52; N 14.28. $C_{28}H_{37}N_5O_3$. Calculated, %: C 68.43; H 7.54; N 14.26.

4-[(Adamantan-1-yl)phenyl]-3-{1-[2-(morpholin-4-yl)-2-oxoethyl]-1H-pyrazol-4-yl}urea (1g). Yield 412 mg (89%). IR (ν, cm^{-1}): 3324, 2901, 2848, 1636, 1596, 1568, 1450, 1400, 1252, 1119, 1041, 645. ^{1}H NMR (DMSO-d6, δ, ppm J, Hz): 8.03 (1H, s, H-5 Pz); 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.62 (1H, s, H-3 Pz); 7.29 (1H, s, NH); 5.98 (1H, br s, NH Ar); 5.02 (2H, s, $NCH_2C(O)$); 3.59 (4H, s, $2OCH_2CH_2N$); 3.48 (2H, s, OCH_2CH_2N); 3.46 (2H, s, OCH_2CH_2N); 1.94 (3H, br s, CH Ad); 1.71–1.58 (6H, m, CH_2 Ad); 1.44 (6H, s, CH_2 Ad). ^{13}C NMR (DMSO-

d6, δ, ppm): 165.7 (C=O); 155.4 (C=O); 129.4 (CH Pz); 123.0 (C-4 Pz); 120.4 (CH Pz); 65.9 (OCH_2); 52.7 (CH_2); 50.9 (CH_2); 44.8 (CH_2); 41.8 (CH_2); 39.7 (CH_2 Ad); 36.6 (CH_2 Ad); 33.5 (C Ad); 27.7(CH Ad). MS (*m/z* (Irel, %): 463 $[M]^{\bullet+}$ (100). Found, %: C 67.37; H 7.19; N 15.13. $C_{26}H_{33}N_5O_3$. Calculated, %: C 67.39; H 7.13; N 15.12.

4-[(Adamantan-1-yl)phenyl]-3-(1-benzyl-1H-pyrazol-3-yl)urea (1h). Yield 391 mg (92%). M.p. 169–171 °C. IR (ν, cm^{-1}): 3360, 3281, 3245, 3137, 3122, 2901, 2848, 1673, 1639, 1573, 1552, 1533, 1453, 1407, 1355, 1345, 1312, 1295, 759, 716. ^{1}H NMR (DMSO-d6, δ, ppm, J, Hz): 8.76 (1H, s, H-5 Pz); 8.69 (1H, s, H-4 Pz); 7.7 (2H, d, J = 7.7, H Ar); 6.6 (2H, d, J = 7.8, H Ar); 7.31 (3H, t, J = 6.8, H Ph); 7.12 (2H, d, J = 6.9, H Ph); 7.01 (1H, br s, NH); 6.05 (1H, br s, NH Ar); 5.18 (2H, s, NCH_2Ph); 1.93 (3H, br s, CH Ad); 1.70–1.52 (6H, m, CH_2 Ad); 1.40 (6H, s, CH_2 Ad). ^{13}C NMR (DMSO-d6, δ, ppm): 159.8; 155.9 (C=O); 150.3; 138.8; 132.2 (CH Ph); 129.6 (CH Ph); 128.7 (CH Ph); 95.3; 55.6 (CH_2); 52.1 (CH_2); 40.9 (CH_2 Ad); 37.7 (CH_2 Ad); 34.5 (C Ad); 28.8 (CH Ad). MS (*m/z* (Irel, %): 426 $[M]^{\bullet+}$ (100). Found, %: C 76.02; H 7.09; N 13.13. $C_{27}H_{30}N_4O$. Calculated, %: C 76.06; H 7.04; N 13.15.

General procedure for the synthesis of aminopyrazoles 3b–h by reduction of nitropyrazoles 4b–h.

At room temperature, 3.2 mmol of nitropyrazole 4b–h is added to a solution of 0.48 mL (9.6 mmol) of $N_2H_4{\bullet}H_2O$ in 30 mL of MeOH, followed by the addition of 22 mg (0.08 mmol) of $FeCl_3{\cdot}6H_2O$ and 160 mg (13.2 mmol) of activated carbon. The reaction mixture is brought to a boil, refluxed for 10 h, and cooled. The precipitate is filtered off, washed 3 × 20 mL with EtOH, the organic fractions are combined, and the solvent is evaporated under reduced pressure. The resulting residue is dissolved in 5 mL of H_2O and acidified with an aqueous HCl solution to pH 3–4. The resulting precipitate is filtered off, washed with 2 ml of cold H_2O and air-dried.

3,5-Dimethyl-1-(4-methylbenzyl)-1H-pyrazol-4-amine (3b). Yield 551 mg (80%), white powder. M.p. 108–109 °C. IR (ν, cm^{-1}): 3377, 3275, 3205, 2920, 1629, 1595, 1515, 1474, 1426, 1374, 1359, 1325, 1255, 1233, 1116, 830, 797, 675, 471. ^{1}H NMR (DMSO-d6, δ, ppm (J, Hz): 7.10 (2H, d, J = 7.6, H Ar), 6.92 (2H, d, J = 7.7, H Ar), 5.05 (2H, s, NCH_2), 3.38 (2H, br. s, $NH_2{+}H_2O$); 2.23 (3H, s, 4-$CH_3C_6H_4$); 2.04 (6H, s, $2CH_3$). MS (*m/z* (Irel, %): 215 $[M]^{\bullet+}$ (100). Found, %: C 72.35; H 8.06; N 19.68. $C_{13}H_{17}N_3$. Calculated, %: C 72.52; H 7.96; N 19.52.

3,5-Dimethyl-1-(4-chlorobenzyl)-1H-pyrazol-4-amine (3c). Yield 581 mg (77%), cream powder. M.p. 62–63 °C. IR (ν, cm^{-1}): 3382, 3269, 3199, 1627, 1595, 1509, 1492, 1475, 1425, 1375, 1361, 1324, 1255, 1098, 1018, 811, 797, 672, 484, 438. ^{1}H NMR (DMSO-d6, δ, ppm (J, Hz): 7.38 (2H, d, J = 8.3, H Ar), 7.02 (2H, d, J = 8.4, H Ar), 5.06 (2H, s, NCH_2), 3.38 (br. s, $NH_2{+}H_2O$); 2.00 (6H, s, $2CH_3$). MS (*m/z* (Irel, %): 235 $[M(^{35}Cl)]^{\bullet+}$ (100), 237 $[M(^{37}Cl)]^{\bullet+}$ (33). Found, %: C 61.19; H 6.04; N 17.93. $C_{12}H_{14}ClN_3$. Calculated, %: C 61.15; H 5.99; N 17.83.

3,5-Dimethyl-1-(4-fluorobenzyl)-1H-pyrazol-4-amine (3d). Yield 519 mg (74%), light-brown powder. M.p. 45–46 °C. IR (ν, cm^{-1}: 3385, 3333, 1605, 1510, 1476, 1439, 1380, 1320, 1252, 1159, 1114, 949, 822, 760, 666, 520, 481. ^{1}H NMR (DMSO-d6, δ, ppm (J, Hz): 7.15–7.05 (4H, m, H Ar); 5.06 (2H, s, NCH_2); 3.38 (br. s, $NH_2{+}H_2O$); 1.98 (6H, s, $2CH_3$) (DMSO-d6, δ, ppm) Hz): 161.3 (d, J = 242.6, C-4' Ar); 136.9; 134.7 (d, J = 2.9, C-1' Ar); 128.7 (d, J = 8.2, C-2',6' Ar); 125.3; 124.6; 115.2 (d, J = 21.3, C-3',5' Ar); 51.1 (NCH_2); 10.9 (CH_3); 8.5 (CH_3). MS (*m/z* (Irel, %): 219 $[M]^{\bullet+}$ (100). Found, %: C 65.80; H 6.42; N 19.29. $C_{12}H_{14}FN_3$. Calculated, %: C 65.73; H 6.44; N 19.16.

General method for the synthesis of nitropyrazolylacetic acid amides 4f,g. 7.5 Mmol of acid 5f,g is added to 25 mL of CCl_4, followed by dropwise addition of 1.1 mL (15 mmol) of $SOCl_2$. The reaction mixture is refluxed for 10 h, and the solvent is removed under reduced pressure. The resulting solid acid chloride residue is dried over P_2O_5 under reduced pressure and used without further purification. 1.3 mL (15 mmol) of morpholine.

3,5-Dimethyl-1-(2-chlorobenzyl)-1H-pyrazol-4-amine (3e). Yield 543 mg (72%), white powder. M.p. 98–99 °C. IR (ν, cm^{-1}): 3346, 3297, 3211, 1591, 1574, 1472, 1445, 1390, 1352, 1331, 1255, 1116, 1048, 1036, 797, 754, 748, 683. ^{1}H NMR (DMSO-d6, δ, ppm (J, Hz): 7.47 (1H, d, J = 6.5, H Ar); 7.32–7.19 (2H, m, H Ar); 6.50 (1H, d, J = 7.0, H Ar); 5.12 (2H, s, NCH_2); 3.61 (br. s, $NH_2{+}H_2O$); 2.03 (6H, s, 2CH3). MS (*m/z* (Irel, %): 235 $[M(^{35}Cl)]^{\bullet+}$ (100), 237 $[M(^{37}Cl)]^{\bullet+}$ (33). Found, %: C 61.07; H 5.91; N 17.88. $C_{12}H_{14}ClN_3$. Calculated, %: C 61.15; H 5.99; N 17.83.

2-(4-Amino-3,5-dimethyl-1H-pyrazol-1-yl)-1-(morpholin-4-yl)ethan-1-one (3f). Yield 503 mg (66%), light yellow powder. M.p. 121–122 °C. IR (ν, cm^{-1}): 3382, 3331, 3201, 2973, 2920, 2866, 1655, 1477, 1445, 1352, 1275, 1242, 1116, 1038, 962, 916, 842, 791, 631, 569. ^{1}H NMR (DMSO-d6, δ, ppm(J, Hz): 4.81 (2H, s, $NCH_2C(O)$); 3.59–3.42 (10H, m, NH_2, $2OCH_2CH_2N$); 1.9 (3H, s, CH3); 1.96 (3H, s, CH_3). ^{13}C NMR (DMSO-d6, δ, ppm: 166.0 (C=O); 136.9 (C Pz); 126.1 (C Pz); 123.8 (C Pz); 66.1 (CH_2); 66.0 (CH_2); 50.2 (CH_2); 44.9 (CH_2); 41.8 (CH_2); 11.0 (CH_3); 8.5 (CH_3). MS (*m/z* (Irel, %): 238 $[M]^{\bullet+}$ (100). Found, %: C 55.30; H 7.70; N 23.74. $C_{11}H_{18}N_4O_2$. Calculated, %: C 55.44; H 7.61; N 23.51.

2-(4-Amino-1H-pyrazol-1-yl)-1-(morpholin-4-yl)-ethan-1-one (3g). Yield 457 mg (68%), light-brown powder. M.p. 149–150 °C. IR (ν,cm^{-1}): 3406, 3344, 3241, 3123, 2955, 2856, 1649, 1594, 1459,1410, 1360, 1269, 1241, 1113, 1039, 1019, 997, 955, 850, 818, 768, 646, 630, 591, 565. ^{1}H NMR (DMSO-d6, δ, ppm (J, Hz): 6.97 (1H, s, H-5 Pz); 6.91 (1H, s, H-3 Pz); 4.89 (2H, s, NCH_2 C(O)); 3.55–3.33 (10H, m, NH_2, $2OCH_2CH_2N$). ^{13}C NMR (DMSO-d6, δ, ppm): 166.0 (C=O); 131.0 (C-4 Pz); 129.7 (CH Pz); 117.8 (CH Pz); 66.0 (CH_2); 59.9 (CH_2); 52.7 (CH_2); 44.9 (CH_2); 41.8 (CH_2). MS (*m/z* (Irel, %): 210 $[M]^{\bullet+}$. Found, %: C 51.49; H 6.67; N 26.72. $C_9H_{14}N_4O_2$. Calculated, %: C 51.42; H 6.71; N 26.65.

1-Benzyl-1H-pyrazol-3-amine (3h). Yield 421 mg (76%), white powder. M.p. 64–65 °C. IR (ν, cm^{-1}): 3430, 3274, 3191, 3095, 3068, 3026, 2920, 1618, 1545, 1499, 1456, 1438, 1394, 1359, 1243, 1193, 1057, 990, 745, 730, 696, 658, 597, 582. ^{1}H NMR (DMSO-d6, δ,

ppm (J, Hz): 7.43 (1H, d, J = 2.0, H-5 Pz); 7.34–7.23 (3H, m, H Ph); 7.18 (2H, d, J = 7.1, N Ph); 5.44 (1H, d, J = 2.0, H-4 Pz); 5.03 (2H, s, NCH_2); 4.58 (2H, s, NH_2). ^{13}C NMR (DMSO-d6, δ, ppm): 155.7 (C-3 Pz); 138.4 (C Ph); 130.7 (C-5 Pz); 128.3 (CH Ph); 127.4 (CH Ph); 127.2 (CH Ph); 92.1 (C-4 Pz); 54.2 (NCH_2). MS (*m/z* (Irel, %): 173 $[M]^{\bullet+}$ (100). Found, %: C 69.20; H 6.44; N 24.36. $C_{10}H_{11}N_3$. Calculated, %: C 69.34; H 6.40; N 24.26.

is added to a suspension of 7.5 mmol of the corresponding acid chloride in 15 mL of anhydrous MeCN. The mixture is stirred at room temperature for 6 h. The precipitate is filtered off, washed with 1 mL of MeCN, and crystallized from H_2O.

2-(3,5-Dimethyl-4-nitro-1H-pyrazol-1-yl)-1-morpholinoethan-1-one (4f). Yield 1.51 g (75%), white powder. M.p. 197–199 °C. IR (ν, cm^{-1}): 3423, 2974, 2864, 1648, 1570, 1496, 1469, 1421, 1376, 1363, 1275, 1242, 1116, 1070, 1041, 1005, 854, 795, 609, 573. ^1H NMR (DMSO-d6, δ, ppm): 5.24 (2H, s, $NCH_2C(O)$); 3.68–3.54 (4H, m, $2OCH_2CH_2N$); 3.54–3.40 (4H, m, $2OCH_2CH_2N$); 2.46 (3H, s, CH_3); 2.39 (3H, s, CH_3). ^{13}C NMR (DMSO-d6, δ, ppm): 164.0 (C=O); 144.6 (C-3 Pz); 142.5 (C-5 Pz); 130.3 (C-4 Pz); 66.0 (CH_2); 65.9 (CH_2); 51.0 (CH_2); 44.7 (CH_2); 42.9 (CH_2); 13.7 (CH_3); 10.5 (CH_3). MS (*m/z* (Irel, %): 268 $[M]^{\bullet+}$ (100). Found, %: C 49.32; H 6.06; N 21.02. $C_{11}H_{16}N_4O_4$. Calculated, %: C 49.25; H 6.01; N 20.88.

1-Morpholino-2-(4-nitro-1H-pyrazol-1-yl)ethan-1-one (4g). Yield 1.39 g (77%), light cream powder. M.p. 160–161 °C. IR (ν, cm-1): 3449, 3127, 2962, 2863, 1655, 1533, 1509, 1474, 1402, 1334, 1307, 1272, 1236, 1218, 1035, 992, 891, 822, 756, 584. ^1H. NMR (DMSO-d6, δ, ppm): 8.77 (1H, s, H-5 Pz); 8.25 (1H, s, H-3 Pz); 5.28 (2H, s, $NCH_2C(O)$); 3.69–3.54 (4H, m, $2OCH_2CH_2N$); 3.54–3.42 (4H, m, $2OCH_2CH_2N$). MS (*m/z* (Irel, %): 240 $[M]^{\bullet+}$ (100). Found, %: C 44.83; H 5.08; N 23.51. $C_9H_{12}N_4O_4$. Calculated, %: C 45.00; H 5.04; N 23.32.

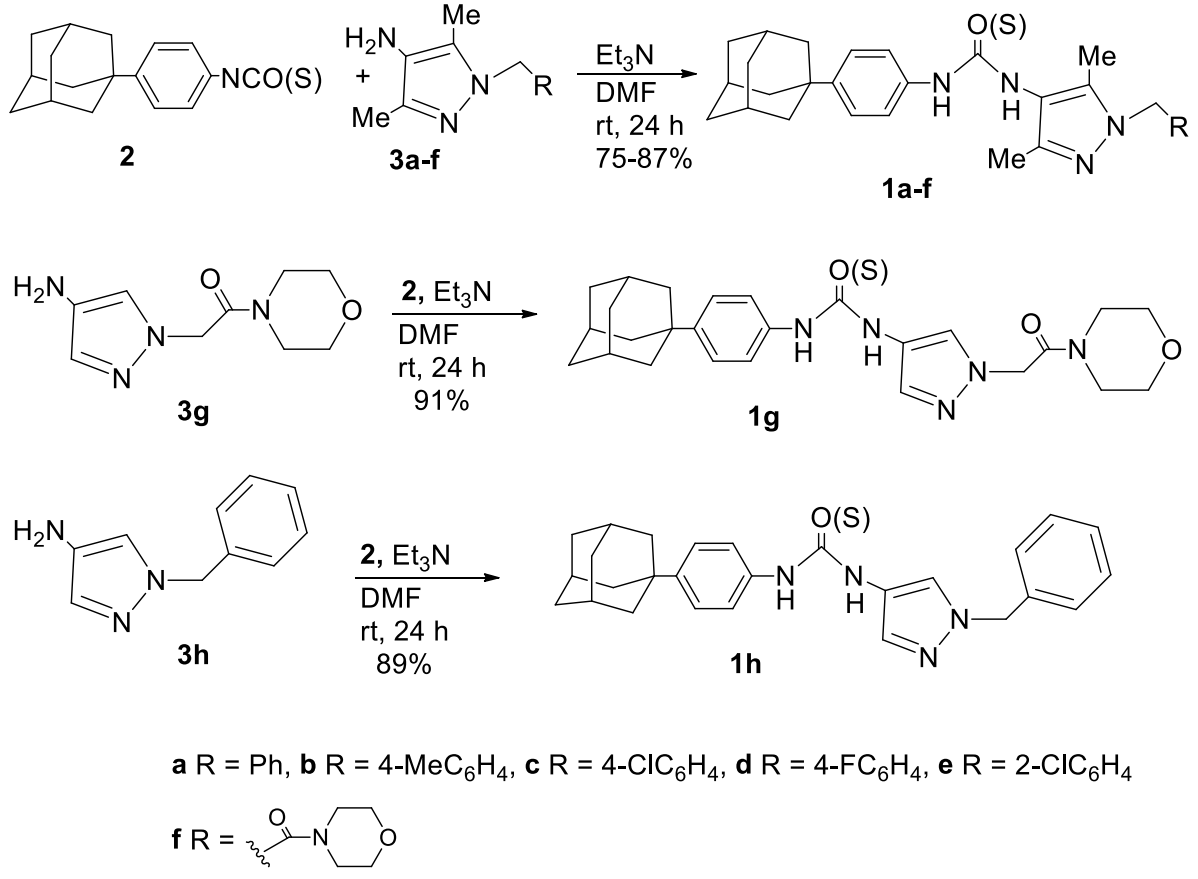
Study of the inhibitory properties of compounds 1a–h. The half-maximal inhibition concentrations (IC50) for human sEH were determined using the described method [28].

To determine the solubility of compounds 1a–e,h in H_2O, series of their solutions in DMSO were prepared with concentrations ranging from 0.5 to 100 mmol/L. Then, 10 μL of the resulting solutions were added to 990 μL of a buffer solution (0.1 mol/L Na_3PO_4, pH 7.4). Thus, the final concentration of the studied compounds ranged from 5 to 1000 μmol/L, and the DMSO concentration was 1%. Solubility was determined by studying the turbidity (adsorption of light with a wavelength of 590 nm) of the resulting aqueous solutions. A buffer solution containing 1% DMSO was used as a reference.

The lipophilicity coefficient cLogP was calculated using the Molinspiration program (www.molinspiration.com).

Results and Discussion

In continuation of the work on the synthesis of adamantyl-containing 1,3-disubstituted ureas with a heterocyclic fragment [21] and the study of their properties, a series of 1,3-disubstituted ureas and thioureas 1a–h containing a pyrazole fragment were obtained for the first time (Scheme 1). The synthesis of products 1a–h was accomplished by the reaction of 1-(4-isocyanatophenyl)adamantane and 1-(4-isothiocyanatophenyl)adamantane (2) with the corresponding aminopyrazoles 3a–h in anhydrous DMF in the presence of Et_3N at a molar ratio of reagents 2:3:Et_3N equal to 1:1:1, stirring for 24 hours at room temperature. During the reaction, precipitation of 1,3-disubstituted ureas and thioureas 1a–h was observed as white crystals. After completion of the reaction, the products were filtered and washed with ethyl acetate, 1 N aqueous HCl to remove residual amine, and a small amount of H_2O. The yields of the obtained compounds 1a–1h were 67–92%. The structure of the obtained compounds was confirmed using IR spectroscopy, 1H and ^{13}C NMR spectroscopy andelementalanalysis.

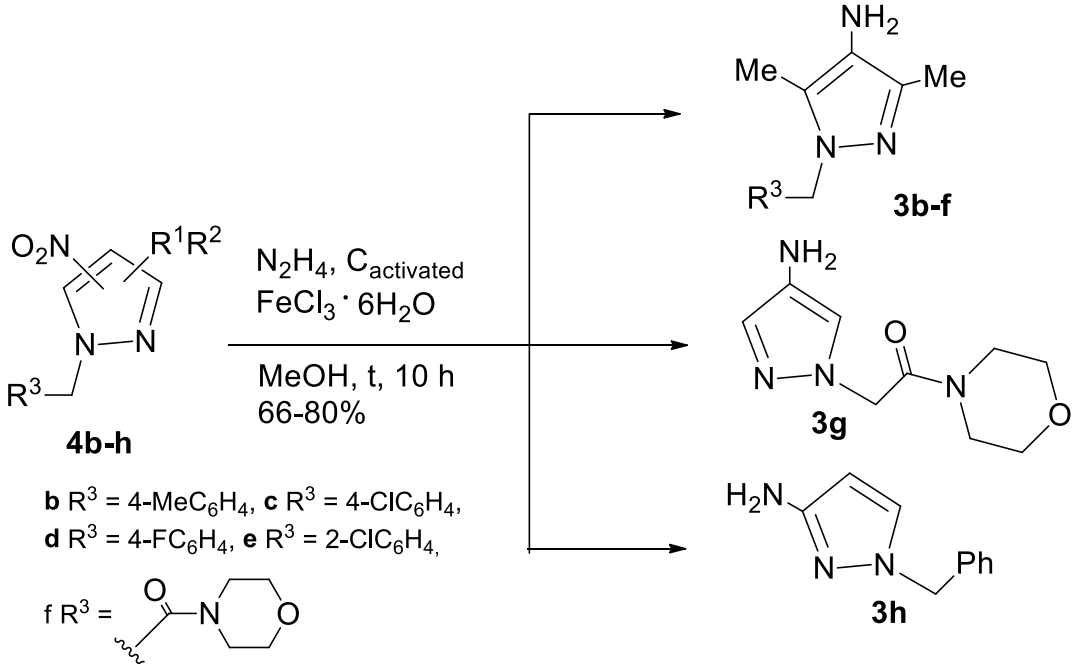


Scheme 1. Synthesis of adamantyl-containing ureas and thioureas containing a pyrazole fragment.

1-(Adamantan-1-yl)-3-(1-alkyl-1H-pyrazolyl)ureas described in the literature were studied as inhibitors of human sEH (IC50 488–2512 nmol/L) and had water solubility values of 4.5–149.3 μmol/L [22]. In order to increase the inhibitory activity against human sEH, the starting reagents for the synthesis of inhibitors were selected. The choice of isocyanate (isothiocyanate) 2 was due to the presence of a phenyl fragment between the isocyanate (isothiophane) group and the adamantyl fragment, which increases the conformational mobility of the pharmacophore fragment and facilitates better adjustment of the inhibitor in the active site of sEH [23]. Benzyl-substituted aminopyrazoles 3a–e,h, as well as aminopyrazoles 3f,g containing the N-[2-(morpholin-4-yl)-2-oxoethyl] group were selected as the pyrazole component (Scheme 1). Such pyrazoles are widely used in the synthesis of biologically active compounds [24]. It is known that the presence of a methyl group, as well as halogen atoms (chlorine, fluorine) in the aromatic ring increases the inhibitory activity of compounds with respect to sEH [19]. In addition, pyrazoles 3a–f contain methyl substituents at positions 3 and 5 of the pyrazole ring, which may help protect it from the oxidative effects of cytochromes P450. The selected series of compounds 3a–h will allow us to study the influence of the structure of substituted pyrazoles on the properties of 1,3-disubstituted ureas (thioureas) 1a–h and their inhibitory activity against human sEH.

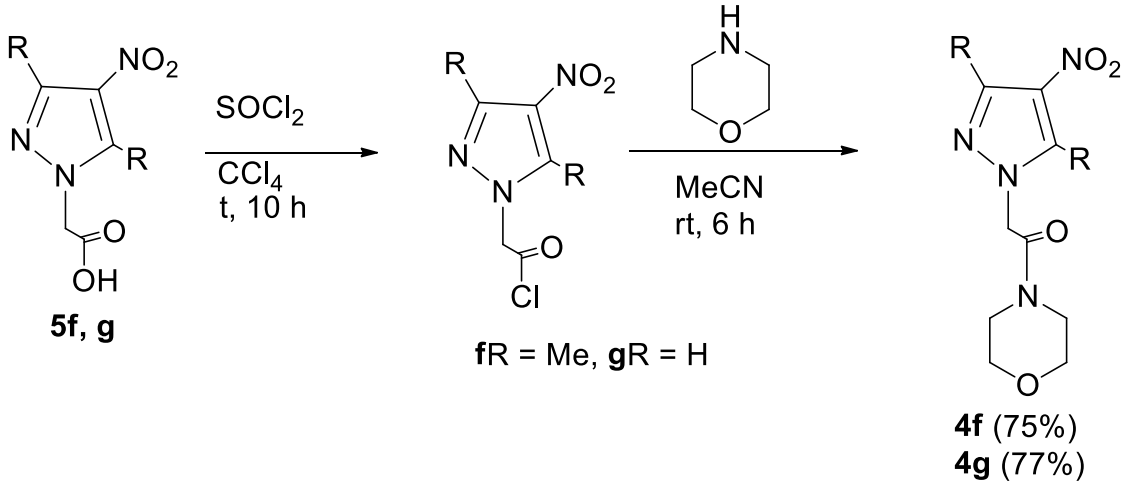
To obtain the starting aminopyrazoles 3b–h, we used the hydrazine reduction method, which has proven itself effective for nitropyrazoles [25]. Indeed, treatment of nitro derivatives 4b–h with hydrazine on activated carbon in the presence of FeCl3•6H2O as a catalyst allowed us to obtain the desired amines 3b–h in high yields

(Scheme 2).



Scheme 2. Synthesis of starting aminopyrazoles 3b-h.

Nitropyrazoles 4f,g with an N-[2-(morpholin-4-yl)-2-oxo-ethyl] substituent were obtained using a known method from previously synthesized nitropyrazolylacetic acids 5f,g [26] (Scheme 3).



Scheme 3. Synthesis of nitropyrazoles 4f,g with N-[2-(morpholin-4-yl)-2-oxo-ethyl] substituent.

The obtained series of 1,3-disubstituted ureas 1a–h were studied as target-oriented sEH inhibitors in vitro. It was found that the inhibitory activity against human sEH for the obtained products 1a–h ranged from 16.2 to 50.2 nmol/L. Other properties of compounds 1a–e,h are presented in Table 1. For compounds 1a–e, the introduction of 4-Me, 4-Cl, 4-F, and 2-Cl substituents into the aromatic ring reduces the solubility in water. Water solubility decreases upon replacement of the fluorine atom with a chlorine atom. The position of the substituent in the aromatic ring (para- or ortho-) has virtually no effect on water solubility. Urea based on unsubstituted pyrazole 1h is less soluble compared to 3,5-dimethylpyrazole-substituted analogs 1a–e. The obtained pyrazole-containing 1,3-disubstituted ureas 1a–e,h are better soluble in water compared to known analogues [22], which suggests their higher bioavailability.

Table 1.

Properties of 1,3-disubstituted ureas (thioureas) 1a–e,h

Compound	Substituents on the benzene ring	Solubility in water, μmol/L	cLogP
1a	-	85±3	4.80(4.96)
1b	4-Me	75±2	5.25(5.57)
1c	4-Cl	65±4	5.48(5.61)
1d	4-F	75±3	4.96(5.03)
1e	2-Cl	65±4	5.43(5.76)
1h	-	45±2	4.35(4.21)

Conclusions

Thus, 1,3-disubstituted ureas and thioureas were synthesized by reacting 1-(isocyanatophenyl)-adamantane and 1-(isothiocyanatophenyl)-adamantane with 3- or 4-aminopyrazoles in anhydrous DMF in the presence of Et3N for 24 hours at room temperature. The high inhibitory activity of the resulting compounds, combined with their increased bioavailability, makes them promising for further studies as human sEH inhibitors.

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