

Supporting information

Synthesis of novel imidazolo-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine hybrids as *In vitro* EGFR inhibitors

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Content

Experimental Section

Spectral data of new compounds

Copies of ¹H-NMR & ¹³C-NMR of selective compounds

Experimental section

Chemistry

All reagents and solvents were obtained from commercial suppliers and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (GF 254) using UV light to visualize the course of the reactions. Melting points were determined using a Cintex apparatus. ¹H and ¹³C NMR spectra were recorded on a Bruker (operating at 400 MHz for ¹H and 100 MHz for ¹³C). Chemical shifts (δ) are reported in ppm with TMS as internal standard. Abbreviations for signal couplings are: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet. Routine monitoring of reaction was performed by TLC using 0.25 mm E. Merck precoated silica gel TLC plates (60 F254). Mass spectra were recorded on a Jeol JMC-300

spectrometer (ESI, 70 eV). Elemental analyses were performed on Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Synthesis of 1-methyl-1H-imidazole-5-carbohydrazide (2): To ethyl 1-methyl-1H-imidazole-5-carboxylate (1) (5 mmol) in methanol (20 ml), 80% hydrazine hydrate (5 mmol) was added in drop wise and the reaction mixture was refluxed about 2h. A white solid separated, which on recrystallization with ethanol gave 2 (78%). ¹H NMR (400 MHz, CDCl₃): δ 9.10 (brs, 1H, -CONH), 7.75 (s, 1H, CH), 7.10 (s, 1H, CH), 4.21 (brs, 2H, -NH₂), 3.55 (s, 3H, CH₃); EI-MS m/z 141.07 [M+H].

Synthesis of 5-(1-methyl-1H-imidazol-5-yl)-1,3,4-oxadiazole-2(3H)-thione (3): To a solution of 2 (3.00 mmol) and carbon disulfide (6.00 mmol) in absolute ethanol (20 ml), potassium hydroxide (3.00 mmol) was added in one portion at 0 °C. The resulting mixture was stirred and refluxed for 8h. The solvent was removed and the residue was acidified with 2M hydrochloric acid and extracted with ethyl acetate (3 × 30 ml). Organic layers were washed with water and dried with anhydrous sodium sulphate (Na₂SO₄). Filtration and concentration in vacuo gave a solid, which was recrystallized from ethanol to give 3 (74%) as pale yellow solid. ¹H NMR (400 MHz, CDCl₃): δ 8.75 (brs, 1H, -NH), 7.73 (s, 1H, CH), 7.08 (s, 1H, CH), 3.53 (s, 3H, CH₃); ESI-MS m/z 183.03[M+H].

Synthesis of 4-amino-3-(1-methyl-1H-imidazol-5-yl)-1H-1,2,4-triazole-5(4H)-thione (4): To a mixture of 3 (3.00 mmol) in ethanol (20 ml), 0.4 ml of 30% hydrazine hydrate was added drop wise and the mixture was refluxed for 6h. After cooling water was added and the mixture was acidified by excess of 3M hydrochloric acid, the separated solid was filtered off, washed with water and crystallized from ethanol to give 4 (70 %) as white solid. ¹H NMR (400 MHz, CDCl₃): δ 9.11 (brs, 1H, -CONH), 7.79 (s, 1H, CH), 7.12 (s, 1H, CH), 3.77 (brs, 2H, -NH₂), 3.52 (s, 3H, CH₃); ESI-MS m/z 197.05 [M+H].

General procedure for the synthesis of 3-(1-methyl-1H-imidazol-5-yl)-6-(aryl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a-5m).

A mixture of 4 (1.50 mmol) and phenacyl bromide (1.50 mmol) in anhydrous ethanol (15 ml) was refluxed for 5h. The solvent was removed under reduced pressure, diethyl ether (20 ml) was added and the reaction mixture was left at 0 °C overnight. The precipitated solid was filtered off, dried and recrystallized with ethanol to give title compounds.

3-(1-methyl-1H-imidazol-5-yl)-6-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5a):

Pale red solid; mp 144-146 °C; ¹H-NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H, CH), 7.68-7.63 (m, 3H, Ar-H), 7.50-7.43 (m, 2H, Ar-H), 7.12 (s, 1H, CH), 3.52 (s, 3H, CH₃), 3.42 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.60, 150.28, 140.53, 135.06, 134.40, 129.92, 128.52(2C), 126.65(2C), 121.22, 111.73, 34.62, 30.55; ESI-MS m/z: 297.08 [M+H]. Anal. Cal for C₁₄H₁₂N₆S; C, 56.74; H, 4.08; N, 28.36; found C, 56.76; H, 4.05; N, 28.39.

3-(1-methyl-1H-imidazol-5-yl)-6-(p-tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5b): Red solid; mp 159-161 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.76 (s, 1H, CH), 7.60 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.42 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.11 (s, 1H, CH), 3.54 (s, 3H, CH₃), 3.41 (s, 2H, -CH₂), 2.33 (s, 3H, Ar-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 151.47, 150.31, 140.25, 139.52, 135.56, 133.24, 128.77(2C), 127.62(2C), 121.29, 111.25, 34.61, 30.87, 21.31; ESI-MS m/z: 311.10 [M+H]. Anal. Cal for C₁₅H₁₄N₆S; C, 58.05; H, 4.55; N, 27.08; found C, 58.06; H, 4.52; N, 28.01.

6-(4-methoxyphenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b]

[1,3,4]thiadiazine (5c): Pale red solid; mp 166-168 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.78 (s, 1H, CH), 7.71 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.12 (s, 1H, CH), 7.00 (d, *J*= 8.0 Hz, 2H, Ar-H), 3.86 (s, 3H, OCH₃), 3.54 (s, 3H, CH₃), 3.42 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 159.32, 151.53, 150.39, 140.49, 135.69, 129.46, 128.12(2C), 121.18, 114.28(2C), 111.29, 56.35, 34.56, 30.42; ESI-MS m/z 327.09 [M+H]; Anal. Cal for C₁₅H₁₄N₆OS: C, 55.20; H, 4.32; N, 25.75; found C, 55.18; H, 4.34; N, 25.78.

6-(4-chlorophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-

b][1,3,4]thiadiazine (5d): Pale red solid; mp 156-158 °C; ¹H NMR (400 MHz, CDCl₃): 7.79 (s, 1H, CH), 7.71 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.52 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.13 (s, 1H, CH), 3.55 (s, 3H, CH₃), 3.43 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.67, 150.38, 140.53, 137.50, 135.49, 134.42, 129.58(2C), 127.78(2C), 121.28, 111.19, 34.21, 30.60; ESI-MS m/z 331.05 [M+H].; Anal. Cal for C₁₄H₁₁ClN₆S: C, 50.83; H, 3.35; N, 25.41; found C, 50.80; H, 3.32; N, 25.44.

6-(4-bromophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b]

[1,3,4]thiadiazine (5e): White solid; mp 163-165 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H, CH), 7.66-7.58 (m, 4H, Ar-H), 7.10 (s, 1H, CH), 3.54 (s, 3H, CH₃), 3.41 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.62, 150.28, 140.67, 135.37, 133.87, 132.19(2C), 128.83(2C), 125.53, 121.14, 111.52, 34.39, 30.44.; ESI-MS m/z: 374.99 [M+H] & 375.99 [M+H]. Anal. Cal for C₁₄H₁₁BrN₆S: C, 44.81; H, 2.95; N, 22.40; found: C, 44.83; H, 2.91; N, 22.44.

4-(3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-yl)

benzonitrile (5f): White solid; mp 177-179 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.76 (s, 1H, CH), 7.68 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.48 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.13 (s, 1H, CH), 3.54 (s, 3H, CH₃), 3.44 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.68, 150.29, 140.65, 136.85, 135.04, 132.81(2C), 130.60(2C), 121.14, 118.81, 116.64, 111.27, 34.48, 30.69.; ESI-MS *m/z*: 322.08 [M+H]. Anal. Cal for C₁₅H₁₁N₇S: C, 56.06; H, 3.45; N, 30.51; found: C, 56.08; H, 3.42; N, 30.55.

3-(1-methyl-1H-imidazol-5-yl)-6-(4-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine

(5g): Pale yellow solid; mp 169-171 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.48 (d, *J*= 8.0 Hz, 2H, Ar-H), 8.25 (d, *J*= 8.0 Hz, 2H, Ar-H), 7.80 (s, 1H, CH), 7.13 (s, 1H, CH), 3.56 (s, 3H, CH₃), 3.44 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.77, 150.42, 148.21, 142.80, 140.40, 135.53, 128.84(2C), 125.72(2C), 121.06, 111.49, 34.57, 30.58.; ESI-MS *m/z*: 342.07 [M+H]. Anal. Cal for C₁₄H₁₁N₇O₂S: C, 49.26; H, 3.25; N, 28.72; found C, 49.23; H, 3.22; N, 28.76.

6-(3-chlorophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b]

[1,3,4]thiadiazine (5h): White solid; mp 185-187 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H, CH), 7.69 (s, 1H, CH), 7.55-7.45 (m, 3H, Ar-H), 7.13 (s, 1H, CH), 3.53 (s, 3H, CH₃), 3.43 (s, 2H, -CH₂); ESI-MS *m/z*: 331.05 [M+H]. Anal. Cal for C₁₄H₁₁ClN₆S: C, 50.83; H, 3.35; N, 25.41; found C, 50.80; H, 3.31; N, 25.43.

3-(1-methyl-1H-imidazol-5-yl)-6-(3-nitrophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine

(5i): Pale red solid; mp 154-156 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.49 (s, 1H, CH), 8.30 (d, *J*= 4.0 Hz, 1H, Ar-H), 7.90-7.84 (m, 2H, Ar-H), 7.76 (s, 1H, CH), 7.11 (s, 1H, CH), 3.53 (s, 3H, CH₃), 3.43 (s, 2H, -CH₂); ESI-MS *m/z*: 342.07 [M+H]. Anal. Cal for C₁₄H₁₁N₇O₂S: C, 49.26; H, 3.25; N, 28.72; found C, 49.24; H, 3.21; N, 28.75.

3-(1-methyl-1H-imidazol-5-yl)-6-(*m*-tolyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (5j):

Pale yellow solid; mp 175-177 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H, CH), 7.64-7.60 (m, 3H, Ar-H), 7.43 (s, 1H, Ar-H), 7.10 (s, 1H, CH), 3.53 (s, 3H, CH₃), 3.43 (s, 2H, -CH₂), 2.37 (s, 3H, Ar-CH₃); ESI-MS *m/z*: 311.10 [M+H]. Anal. Cal for C₁₅H₁₄N₆S: C, 58.05; H, 4.55; N, 27.08; found: C, 58.02; H, 4.58; N, 27.09.

6-(3-methoxyphenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-

b][1,3,4]thiadiazine (5k): Yellow solid; mp 180-182 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.77 (s, 1H, CH), 7.69-7.63 (m, 2H, Ar-H), 7.50-7.46 (m, 1H, Ar-H), 7.13 (s, 1H, CH), 7.01 (s, 1H, ArH),

3.85 (s, 3H, OCH₃), 3.52 (s, 3H, CH₃), 3.41 (s, 2H, -CH₂); ESI-MS m/z: 327.09 [M+H].
Anal. Cal for C₁₅H₁₄N₆OS; C, 55.20; H, 4.32; N, 25.75; found C, 55.21; H, 4.35; N, 25.79.

6-(3-bromophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-b]

[1,3,4]thiadiazine (5l): White solid; mp 163-165 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.78 (s, 1H, CH), 7.69 (s, 1H, Ar-H), 7.60-7.53 (m, 3H, Ar-H), 7.11 (s, 1H, CH), 3.53 (s, 3H, CH₃), 3.42 (s, 2H, -CH₂); ESI-MS m/z: 374.99 [M+H] & 376.99 [M+3H]. Anal. Cal for C₁₄H₁₁BrN₆S: C, 44.81; H, 2.95; N, 22.40; found: C, 44.76; H, 2.92; N, 22.43.

6-(3,5-dinitrophenyl)-3-(1-methyl-1H-imidazol-5-yl)-7H-[1,2,4]triazolo[3,4-

b][1,3,4]thiadiazine (5m): White solid; mp 168-170 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.60 (s, 1H, Ar-H), 8.30 (s, 2H, Ar-H), 7.81 (s, 1H, CH), 7.13 (s, 1H, CH), 3.54 (s, 3H, CH₃), 3.43 (s, 2H, -CH₂); ¹³C NMR (100 MHz, CDCl₃) δ 151.60, 150.29, 146.86(2C), 140.69, 138.50, 135.32, 126.11(2C), 124.15, 121.38, 111.30, 34.63, 30.52.; ESI-MS m/z: 387.05 [M+H]. Anal. Cal for C₁₄H₁₀N₈O₄S: C, 43.52; H, 2.61; N, 29.00; found: C, 43.47; H, 2.56; N, 29.08.











