Synthesis, Crystal Structure and Antiproliferative Activity of 6-Adamantyl-3-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles

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A series of 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a** – **l** bearing an adamantyl moiety were synthesized by condensation of 4-amino-5-aryl-2*H*-1,2,4-triazole-3(4*H*)-thiones **4a** – **l** with adamantyl-1-carboxylic acid in the presence of $POCl_3$. The structures of the newly synthesized compounds were established using spectroanalytical techniques and verified further by the crystal structure determination of compounds **5a** and **5j**. The compounds were screened for their antiproliferative activity against a large panel of human cell lines.

Key words: Antiproliferative Activity, [1,2,4]Triazolo[3,4-*b*][1,3,4]thiadiazoles, Adamantyl Derivatives

Introduction

Fused heterocycles have received significant attention due to their synthetic and biological importance, and 1,2,4-triazole-3-thiones/thiols are excellent candidates for their synthesis. A number of significant activities, like antitumor and antimicrobial, have been associated with these condensed heterocycles [1 – 6]. Moreover, among various substituents, adamantyl derivatives are gaining importance in well known drugs like Rimantadine, Memantine, Adapalene, Adatanserine and others in clinical trials [7, 8]. The adamantyl derivatives are also recognized for their activity against influenza [9] and HI viruses [10]. Some other adamantyl derivatives have been used as anti-inflammatory [11, 12], antimicrobial [13, 14], antimalarial [15], and antidepressant agents [16] as well as inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [17].

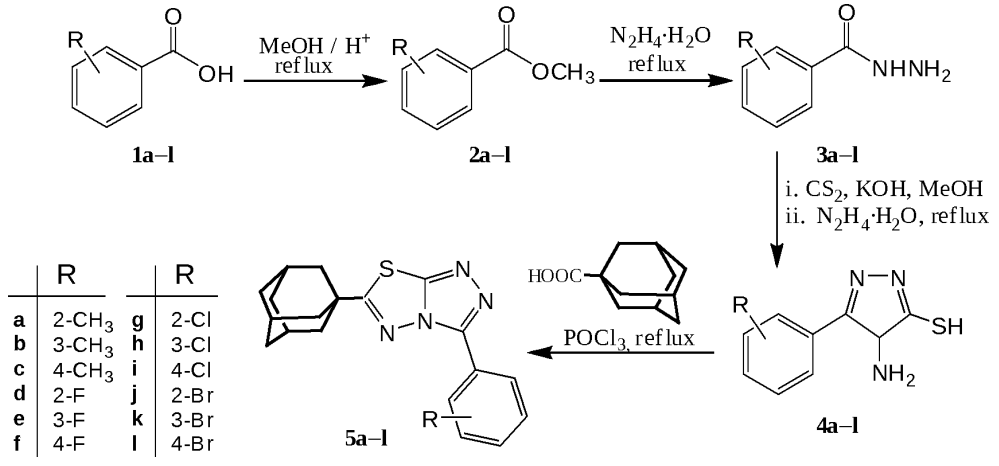
In continuation of our work on the synthesis and biological evaluation of five-membered heterocycles [18a – f], we have synthesized triazolothiadiazoles bearing an adamantyl moiety and evaluated their antiproliferative activities against a large panel of human cell lines.

Results and Discussion

Synthesis

The synthesis of the adamantyl-bearing 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a** – **l** was carried out by treatment of 4-amino-5-aryl-2*H*-1,2,4-triazole-3(4*H*)-thiones **4a** – **l** with adamantyl-1-carboxylic acid [6]. 4-Amino-5-aryl-2*H*-1,2,4-triazole-3(4*H*)-thiones were prepared from aryl hydrazides **3a** – **l** by reaction with CS_2 in the presence of KOH, followed by cyclization with hydrazine hydrate [18a]. The aryl hydrazides **3a** – **l** were synthesized from the corresponding aryl benzoic acids **1a** – **l** *via* esterification to **2a** – **l**, by following a reported procedure [18f]. The synthetic steps are illustrated in Scheme 1.

The condensation of **4a** – **l** with adamantyl-1-carboxylic acid to **5a** – **l** was achieved in 64 – 84 % yield. The structures of **5a** – **l** were confirmed by ^{1}H- and ^{13}C-NMR, IR and mass spectra. The cyclization of **4a** – **l** to [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a** – **l** was followed by the IR and ^{1}H-NMR spectra, where the NH/NH_2 signals disappeared in comparison to triazolethiones **4a** – **l**. In the ^{1}H-NMR spectra of **5a** – **l**, the adamantane protons resonated in the region δ =



Scheme 1. Synthesis of 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles.

1.85 – 2.21 ppm as multiplets. Four aromatic protons exhibited multiplicities depending on the position of the substituent on the benzene ring. In addition, the aromatic proton signals in **5d** – **f** exhibited splittings due to proton-fluorine coupling. In the ^{13}C-NMR spectra, adamantyl carbons appeared in the region δ = 21.2 – 28.5 ppm, whereas the signals in the aromatic region of δ = 123.0 – 140.0 ppm varied from four to six due to different substitution patterns on the aryl ring. The doublets at δ = 159.9, 162.8 and 163.8 ppm with large $J_{C,F}$ values (254.2, 244.5, and 249.0 Hz) were assigned to C-2, C-3 and C-4 of the aromatic residues in compounds **5d**, **5e**, and **5f**, respectively. The signal of C-8 of the triazolothiadiazole unit was observed in the region δ = 142.9 – 149.9 ppm, whereas C-3-triazole resonated in the region of 153.2 – 154.8 ppm. The higher-field signals between δ = 179.4 and 182.3 ppm were assigned to C-6-S.

The constitution of compounds **5a** – **l** was further confirmed by the single crystal X-ray studies of compounds **5a** and **5j** (Figs. 1 and 2). Compound **5j** crystallizes with four independent molecules in the asymmetric unit, all of which differ in molecular conformation. Molecular dimensions may be regarded as normal. The heterocyclic ring systems are planar within mean deviations of 0.01 Å. The relative ring orientations are defined (i) by the interplanar angles between the heterocycle and the phenyl rings (26.3° for **5a**, 52.5, 57.0, 52.0, 55.7° for **5j**) together with the torsion angles N2–C1–C4–C5 (29.0°) for **5a**, N2–C1–C4–C9 (48.4, 52.4, −47.9, −52.3°) for **5j**, and (ii) the relative orientation of the heterocycle and the adamantyl system, as indicated *e. g.* by the smallest absolute torsion angle

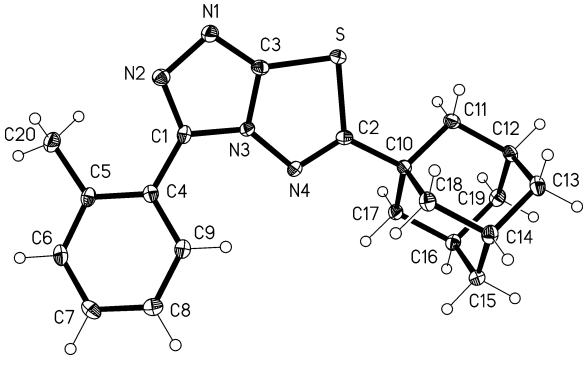


Fig. 1. Structure of compound **5a** in the crystal. Displacement ellipsoids at the 50 % probability level.

S–C2–C10–C (−14.6° for **5a**, −27.6, 42.7, −28.1, 14.9° for **5j**, all involving C11 as the fourth atom).

In vitro antiproliferative activity

Compounds **5a** – **l** were tested *in vitro* against a large panel of human cell lines derived from hematological CD4$^+$ human T cells containing an integrated HTLV-1 genome (MT-4), CD4$^+$ human acute T-lymphoblastic leukaemia (CCRF-CEM), human splenic B-lymphoblastoid cells (WIL-2NS), human acute B-lymphoblastic leukemia (CCRF-SB) and solid skin melanoma (SK-28), breast adenocarcinoma (MCF-7), lung squamous carcinoma (SK-MES-1), hepatocellular carcinoma (HepG-2), prostate carcinoma (DU-145) or normal tissues [lung fibroblasts (MRC-5)]. The Microculture Tetrazolium Assay (MTT) method [19] was used for estimation of the *in vitro* tumor-inhibiting activity of the tested

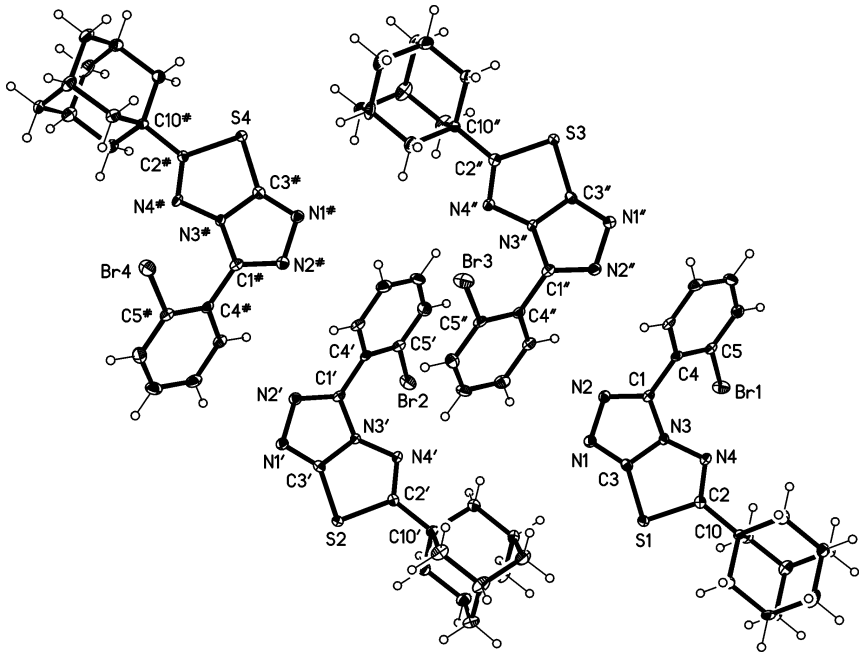


Fig. 2. Structure of compound **5j** (four independent molecules) in the crystal. Displacement ellipsoids at the 50 % probability level.

compounds. The cell lines of tumor subpanels were incubated within five concentrations (0.01 – 100 g mL^{-1}) of each test compound for 48 h. For comparative purposes, we evaluated the cytotoxic activities of the compounds relative to Doxorubicin.

All compounds were inactive except **5a** and **5d** which showed activity against human $CD4^+$ T cells containing an integrated HTLV-1 genome (CD^+) (CC_{50} = 45 μM and 47 μM, respectively) and human acute T-lymphoblastic leukemia (CCRF-CEM) cell lines (CC_{50} = 40 μM, for both compounds). Furthermore, compounds **5a** and **5d** exhibited activity against human acute B-lymphoblastic leukaemia (CCRF-SB) lines (CC_{50} = 50 μM and 48 μM, respectively) and human splenic lymphoblastoid (WIL-2NS) lines (CC_{50} = 52 μM and 44 μM, respectively).

Conclusion

A series of 3,6-disubstituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles **5a** – **l** were synthesized and characterized by spectral techniques. The structures of **5a** and **5j** were further confirmed by single crystal X-ray diffraction studies. Compounds **5a** and **5d** exhibited antiproliferative activity against some selected human cell lines. Introduction of methyl or fluoro residues in the *ortho* position of the aromatic ring of **5** generally enhanced the potency: dramatic changes in activity were observed with the other congeners (compounds **5b**, **c**, **5e** – **l**). Thus, substitution of the aromatic ring by chloro or bromo residues at *ortho*, *meta* or *para* positions (**5g** – **l**) did not show any activity. Similary, 3- or 4-methylphenyl (**5b**, **c**), or 3- or 4-fluorophenyl groups (**5e**, **f**) exhibited no activity as well.

Experimental Section

General

Melting points were measured using a Stuart SMP3 melting point apparatus and are uncorrected. The R_f values were determined using pre-coated silica gel aluminium plates with silica gel 60 F_{254} (Merck, Germany). Column chromatography was carried out using silica gel 60 (0.063 – 0.200 mm) obtained from Merck, Germany. The IR spectra were recorded on an FTS 3000 MX, Bio-Rad Merlin spectrometer (Excalibur Model). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 MHz-NMR spectrometer and signals calibrated to the residual signals of the solvent. The EI mass spectra were carried out using an Agilent Technologies 6890N (GC) mass spectrometer and an inert selective detector 5973. 4-Amino-5-aryl-2*H*-1,2,4-triazole-3(4*H*)-thiones **4a** – **l** were prepared according to a reported procedure [19].

*General procedure for the synthesis of 3,6-disubstituted [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (**5a** – **l**)*

A mixture of the respective triazolethione **4a** – **l** (0.90 mmol) and adamantane-1-carboxylic acid (0.90 mmol)

was heated under reflux for 4 h in the presence of $POCl_3$ (5.0 mL). The reaction mixture was cooled to r. t., poured on ice and neutralized with solid K_2CO_3 until pH = 8. The precipitated solid was filtered, washed with excess of water and purified by column chromatography (*n*-hexane : ethyl acetate 7 : 3).

*6-(1-Adamantyl)-3-(2-methylphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5a**)*

Yield: 76 %; m. p. 196 – 198 °C; R_f: 0.58 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3025, 2905, 2847, 1529, 1452. – ^{1}H-NMR ($CDCl_3$): δ = 1.81 (bs, 6H, adamant.-H), 2.08 (bs, 6H, adamant.-H), 2.15 (bs, 3H, adamant.-H), 2.58 (s, 3H, Ar-*Me*), 7.34 – 7.41 (m, 3H, Ar-H), 7.86 (m, 1H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 21.2, 28.1, 36.1, 39.8, 42.2 (C-adamant.), 125.1, 125.8, 129.5, 130.0, 131.2, 138.1 (C-arom.), 146.9 (C-8), 153.9 (C-3-triazole), 179.4 (C-6-S). – EIMS: *m/z* (%) = 350 (100) $[M]^+$, 215 (12), 135 (27), 117 (28), 107 (17), 93 (21), 79 (31).

*6-(1-Adamantyl)-3-(3-methylphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5b**)*

Yield: 68 %; m. p. 190 – 192 °C; R_f: 0.62 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3034, 2998, 2852, 1558, 1457. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.20 (bs, 3H, adamant.-H), 2.47 (s, 3H, Ar-*Me*), 7.30 (m, 1H, Ar-H), 7.43 (m, 1H, Ar-H), 8.15 – 8.17 (m, 2H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 21.5, 28.1, 36.1, 39.9, 42.3 (C-adamant.), 123.4, 125.7, 126.9, 128.7, 131.0, 138.6 (C-arom.), 146.3 (C-8), 154.4 (C-3-triazole), 179.6 (C-6-S). – EIMS: *m/z* (%) = 350 (100) $[M]^+$, 215 (10), 135 (9), 117 (31), 107 (16), 93 (12), 79 (13).

*6-(1-Adamantyl)-3-(4-methylphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5c**)*

Yield: 80 %; m. p. 184 – 186 °C; R_f: 0. 58 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3033, 2907, 2846, 1540, 1455. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.20 (bs, 3H, adamant.-H), 2.45 (s, 3H, Ar-*Me*), 7.35 (d, 2H, *J* = 8.1 Hz, Ar-H), 8.24 (d, 2H, *J* = 8.1 Hz, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 21.6, 28.1, 36.2, 39.9, 42.3 (C-adamant.), 123.1, 126.2, 129.6, 129.9, 140.0 (C-arom.), 143.2 (C-8), 153.2 (C-3-triazole), 179.5 (C-6-S). – EIMS: *m/z* (%) = 350 (100) $[M]^+$, 215 (16), 135 (13), 117 (42), 107 (10), 93 (13), 79 (16).

*6-(1-Adamantyl)-3-(2-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5d**)*

Yield: 66 %; m. p. 188 – 190 °C; R_f: 0.53 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3029, 2906, 2854, 1540, 1455, 1020. – ^{1}H-NMR ($CDCl_3$): δ = 1.82 (bs, 6H, adamant.-H), 2.10 (bs, 6H, adamant.-H), 2.17 (bs, 3H, adamant.-H), 7.25 – 7.36 (m, 2H, Ar-H), 7.52 (m, 1H, Ar-H), 8.05 (dt, 1H, $J_{H,F}$ = 7.5, 1.8 Hz, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 39.9, 42.2 (C-adamant.), 114.3 (d, J_{C-3F} = 12.7 Hz, C-3-arom.), 116.6 (d, J_{C-1F} = 20.2 Hz, C-1-arom.), 124.4 (d, J_{C-5F} = 3.8 Hz, C-5-arom.), 130.2 (d, J_{C-6F} = 2.2 Hz, C-6-arom.), 132.1 (d, J_{C-4F} = 8.3 Hz, C-4-arom.), 142.9 (C-8), 154.5 (C-3-triazole), 159.9 (d, J_{C-2F} = 254.2 Hz, C-arom.), 179.6 (C-6-S). – EIMS: m/z = 354 (100) $[M]^+$, 135 (11), 121 (34), 107 (9), 93 (7), 79 (13).

*6-(1-Adamantyl)-3-(3-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5e**)*

Yield: 64 %; m. p. 209 – 210 °C; R_f: 0.59 (*n*-hexane: ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3020, 2907, 2848, 1520, 1455, 998. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.21 (bs, 3H, adamant.-H), 7.19 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 8.09 (m, 1H, Ar-H), 8.17 (m, 1H, ArH). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 40.0, 42.3 (C-adamant.), 113.2 (d, J_{C-2F} = 24.0 Hz, C-2-arom.), 117.0 (d, J_{C-4F} = 21.0 Hz, C-4-arom.), 121.9 (d, $J_{C-6,F}$ = 3.0 Hz, C-6-arom.), 127.8 (d, $J_{C-5,F}$ = 9.0 Hz, C-5-arom.), 136.6 (d, $J_{C-1,F}$ = 8.3 Hz, C-1-arom.), 145.1 (C-8), 154.8 (C-3-triazole), 162.8 (d, J_{C-3F} = 244.5 Hz, C-arom.-F), 180.3 (C-6-S). – EIMS: *m/z* (%) = 354 (100) $[M]^+$, 135 (13), 121 (49), 107 (8), 93 (9), 79 (16).

*6-(1-Adamantyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5f**)*

Yield: 70 %; m. p. 198 – 200 °C; R_f: 0.56 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3017, 2909, 2849, 1531, 1458, 1001. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.20 (bs, 3H, adamant.-H), 7.20 – 7.28 (m, 2H, Ar-H), 8.34 – 8.39 (m, 2H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 39.9, 42.3 (C-adamant.), 116.0 (d, J_{C-3F} = $J_{C-5,F}$ = 21.7 Hz, C-3-arom., C-5-arom.), 122.2 (d, J_{C-1F} = 3.0 Hz, C-1-arom.), 128.4 (d, J_{C-2F} = J_{C-6F} = 8.2 Hz, C-2-arom., C-6-arom.), 145.4 (C-8), 154.0 (C-3-triazole), 163.8 (d, J_{C-4F} = 249.0 Hz, C-4-arom.), 180.0 (C-6-S). – EIMS: *m/z* (%) = 354 (100) $[M]^+$, 135 (14), 121 (57), 107 (8), 93 (10), 79 (15).

*6-(1-Adamantyl)-3-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5g**)*

Yield: 84 %; m. p. 219 – 220 °C; R_f: 0.59 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3026, 2907, 2845, 1537, 1449, 1054. – ^{1}H-NMR ($CDCl_3$): δ = 1.80 (bs, 6H, adamant.-H), 2.08 (bs, 6H, adamant.-H), 2.15 (bs, 3H, adamant.-H), 7.41 – 7.51 (m, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 7.79 (m, 1H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.0, 36.1, 39.9, 42.2 (C-adamant.), 125.3, 126.9, 130.5, 131.5, 131.9,

Table 1. Crystal structure data for **5a** and **5j**.

	5a	**5j**
Formula	$C_{20}H_{22}N_4S$	$C_{19}H_{19}BrN_4S$
M_r	350.48	415.35
Crystal size, mm^3	$0.3 \times 0.2 \times 0.2$	$0.3 \times 0.25 \times 0.2$
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$
a, Å	11.0863(3)	18.9854(5)
b, Å	11.6370(3)	18.1514(5)
c, Å	13.2322(4)	20.8524(5)
β, deg	98.664(4)	103.982(4)
V, Å^3	1687.62	6973.1
Z	4	16
D_{calcd}, Mg m^{-3}	1.38	1.58
μ(MoK_α), mm^{-1}	0.2	2.5
F(000), e	744	3392
$2\theta_{max}$, deg	58.3	58.3
Refl. meas. /	35684 /	155223 /
indep. /	4520 /	18653 /
R_{int}	0.045	0.055
Param. refined	227	901
wR (F^2, all refl.)	0.086	0.045
GoF (F^2)	1.004	0.793
Max. $\Delta\rho_{fin}$, e Å^{-3}	0.38	0.67

133.9 (C-arom.), 145.0 (C-8), 154.4 (C-3-triazole), 179.5 (C-6-S). – EIMS: *m/z* (%) = 372 (35) $[M+2]^+$, 370 (100) $[M]^+$, 137 (28), 135 (12), 107 (13), 93 (11), 79 (19).

*6-(1-Adamantyl)-3-(3-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5h**)*

Yield: 82 %; m. p. 208 – 210 °C; R_f: 0.65 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3025, 2901, 2848, 1539, 1457, 997. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.15 (bs, 6H, adamant.-H), 2.21 (bs, 3H, adamant.-H), 7.46 – 7.48 (m, 2H, Ar-H), 8.26 (m, 1H, Ar-H), 8.38 (m, 1H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 40.0, 42.3 (C-adamant.), 124.3, 126.2, 127.5, 130.1, 130.2, 134.9 (C-arom.), 144.0 (C-8), 154.0 (C-3-triazole), 180.3 (C-6-S). – EIMS: *m/z* (%) = 372 (35) $[M+2]^+$, 370 (100) $[M]^+$, 137 (41), 135 (15), 107 (9), 93 (13), 79 (20).

*6-(1-Adamantyl)-3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5i**)*

Yield: 84 %; m. p. 252 – 254 °C; R_f: 0.65 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3063, 2896, 2846, 1543, 1447, 1001. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.21 (bs, 3H, adamant.-H), 7.50 – 7.54 (m, 2H, Ar-H), 8.30–8.33 (m, 2H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 27.6, 36.1, 40.0, 42.0 (C-adamant.), 124.7, 127.5, 129.2, 136.1 (C-arom.), 145.3 (C-8), 154.7 (C-3-triazole), 180.2 (C-6-S). – EIMS: *m/z* (%) = 372 (36) $[M+2]^+$, 370 (100) $[M]^+$, 137 (48), 135 (14), 107 (11), 93 (12), 79 (19).

Table 2. Selected bond lengths (Å), and angles (deg) for **5a** and **5j**.

	5a	**5j**
S–C(3)	1.7295(12)	1.7340(17)
S–C(2)	1.7707(12)	1.7771(17)
N(1)–C(3)	1.3096(14)	1.308(2)
N(1)–N(2)	1.4002(14)	1.4078(18)
N(2)–C(1)	1.3235(15)	1.3154(19)
N(3)–C(3)	1.3583(15)	1.3578(19)
N(3)–N(4)	1.3789(12)	1.3742(17)
N(3)–C(1)	1.3792(14)	1.3683(19)
N(4)–C(2)	1.2976(15)	1.2926(19)
C(1)–C(4)	1.4660(16)	1.466(2)
C(2)–C(10)	1.5043(15)	1.503(2)
C(3)–S–C(2)	87.85(5)	87.68(8)
C(3)–N(1)–N(2)	105.04(9)	104.88(13)
C(1)–N(2)–N(1)	109.72(9)	109.14(12)
C(3)–N(3)–N(4)	118.21(9)	118.99(13)
C(3)–N(3)–C(1)	106.02(9)	105.85(13)
N(4)–N(3)–C(1)	135.70(10)	135.10(14)
C(2)–N(4)–N(3)	108.28(9)	107.97(13)
N(2)–C(1)–N(3)	107.43(10)	108.31(14)
N(2)–C(1)–C(4)	127.95(10)	126.27(14)
N(3)–C(1)–C(4)	124.55(10)	125.12(14)
N(4)–C(2)–C(10)	121.83(10)	121.98(14)
N(4)–C(2)–S	116.26(9)	116.43(12)
C(10)–C(2)–S	121.83(8)	121.45(12)
N(1)–C(3)–N(3)	111.78(10)	111.81(15)
N(1)–C(3)–S	138.81(9)	139.35(14)
N(3)–C(3)–S	109.39(8)	108.85(12)

*6-(1-Adamantyl)-3-(2-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5j**)*

Yield: 74 %; m. p. 217 – 219 °C; R_f: 0.58 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3044, 2913, 2850, 1529, 1452, 643. – ^{1}H-NMR ($CDCl_3$): δ = 1.80 (bs, 6H, adamant.-H), 2.07 (bs, 6H, adamant.-H), 2.14 (bs, 3H, adamant.-H), 7.40 (m, 1H, Ar-H), 7.48 (m, 1H, Ar-H), 7.71 – 7.77 (m, 2H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.5, 36.1, 39.8, 42.2 (C-adamant.), 123.1, 127.4, 127.6, 131.7, 132.3, 133.7 (C-arom.), 146.0 (C-8), 154.3 (C-3-triazole), 179.5 (C-6-S). – EIMS: *m/z* (%) = 416 (98) $[M+2]^+$, 414 (100) $[M]^+$, 281 (8), 279 (8), 183 (16), 181 (16), 135 (20), 107 (18), 93 (25), 79 (32).

*6-(1-Adamantyl)-3-(3-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5k**)*

Yield: 70 %; m. p. 229 – 230 °C; R_f: 0.62 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3035, 2899, 2845, 1540, 1456, 681. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.15 (bs, 6H, adamant.-H), 2.21 (bs, 3H, adamant.-H), 7.42 (m, 1H, Ar-H), 7.62 (m, 1H, Ar-H), 8.31 (m, 1H, Ar-H), 8.54 (m, 1H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 40.0, 42.3 (C-adamant.), 123.0, 124.7, 127.7, 129.1, 130.4, 133.0 (C-arom.), 144.8 (C-8), 154.8 (C-3-triazole), 180.3 (C-6-S). – EIMS: *m/z* (%) = 416 (98)

$[M+2]^+$, 414 (100) $[M]^+$, 281 (5), 279 (5), 183 (34), 181 (34), 135 (30), 107 (15), 93 (24), 79 (36).

*6-(1-Adamantyl)-3-(4-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (**5l**)*

Yield: 76 %; m. p. 252 – 254 °C; R_f: 0.60 (*n*-hexane : ethyl acetate 6 : 4). – IR (KBr, cm^{-1}): ν = 3040, 2907, 2849, 1521, 1447, 679. – ^{1}H-NMR ($CDCl_3$): δ = 1.85 (bs, 6H, adamant.-H), 2.14 (bs, 6H, adamant.-H), 2.21 (bs, 3H, adamant.-H), 7.66 – 7.70 (m, 2H, Ar-H), 8.23 – 8.26 (m, 2H, Ar-H). – ^{13}C-NMR ($CDCl_3$): δ = 28.1, 36.1, 39.9, 42.3 (C-adamant.), 124.5, 124.8, 127.7, 132.1 (C-arom.), 145.4 (C-8), 154.7 (C-3-triazole), 180.3 (C-6-S). – EIMS: *m/z* (%) = 416 (98) $[M+2]^+$, 414 (100) $[M]^+$, 281 (7), 279 (7), 183 (43), 181 (43), 135 (27), 107 (18), 93 (23), 79 (34).

Cytotoxicity assays

Cell cultures were seeded at 1×10^5 cells per mL in 96 multiwell plates in specific media supplemented with 10 % FCS and antibiotics and incubated at 37 °C in a humidified CO_2 (5 %) atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method. Compounds were dissolved in DMSO at 100 mM and then diluted into culture medium.

X-Ray structure determinations

Crystal data and refinement details are presented in Table 1. *Data collection and reduction:* Crystals were mounted in inert oil on glass fibers and transferred to the cold gas stream of an Oxford Diffraction Xcalibur S diffractometer. Measurements were performed with monochromated MoK_α radiation (λ = 0.71073 Å). Absorption corrections were performed on the basis of multi-scans. *Structure refinement*: The structures were refined anisotropically against F^2 (program SHELXL-97) [20]. Methyl groups were refined as idealized rigid groups allowed to rotate but not tip; other hydrogen atoms were included with a riding model. For **5j**, restraints to displacement parameters were employed to improve stability of refinement. Table 2 lists some selected bond lengths and angles.

CCDC 743335 (**5a**) and 743336 (**5j**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

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