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Synthesis and antiviral activity evaluation of some new 6-substituted 3-(1-adamantyl)-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles

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Abstract

A number of novel 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives, containing the adamantyl moiety, were synthesized and examined in various viral test systems. No antiviral effects were noted with any of the compounds at subtoxic concentrations in cell culture. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazoles; Schiff base; Antiviral activity

1. Introduction

Triazoles and their heterocyclic derivatives represent an interesting class of compounds possessing a wide spectrum of biological activities. A large number of triazole-containing ring systems exhibits antifungal [1], antibacterial [2], anticancer [3] and antiviral [4] properties.

In a previous paper we have reported the synthesis of some compounds containing the triazole ring, which possessed moderate antiviral activity [5]. It has also been recently reported that some 3-methyl or benzyl 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives are endowed with anti-HIV-1 activity at subcytotoxic concentrations [6]. In view of these facts and as a continuation of our research on the biological properties of triazole-containing derivatives, we have designed and synthesized a number of adamantyl substituted fused triazole systems, as potential antiviral agents. We have combined the adamantane moiety with the triazole nucleus, since both of these systems possess well-documented antiviral properties [7].

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2. Chemistry

1-Adamantanecarboxylic acid (**1**) was converted, according to a published procedure [8], first to the corresponding ethyl ester (**2**), and then to the hydrazide **3** (Scheme 1). This was heated with carbon disulfide in the presence of absolute ethanol and potassium hydroxide to afford the intermediate potassium acylhydrazine dithioformate (**4**). This salt underwent ring closure with an excess of 95% hydrazine to give the 3-adamantyl-4-amino-5-mercapto-1,2,4-triazole (**5**) [9]. Heating at reflux the triazole **5** with phosphorus oxychloride and the appropriate acyl chloride provided the 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazoles (**6a**–**f**). The structure of these compounds was confirmed by ^{1}H NMR and elemental analysis.

In an attempt to prepare the corresponding 5,6-dihydro analogs, we have applied a previously reported procedure [10] and treated the triazole **5** with the appropriate benzaldehyde to obtain the corresponding Schiff base, which would then undergo an intramolecular nucleophilic attack of the mercapto group on –CH=N–, to provide the ring closed derivatives **8**. Special care was taken to maintain the pH during the reaction at values 5–6, since the acidity of the reaction medium is crucial, as reported previously [10].

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However, with three differently substituted benzaldehydes that we have used to run this reaction under the above mentioned conditions, we could only isolate pure the open chain hydrazones **7a–c**. The formation of these compounds is in accordance with a previous publication [1], concerning a similar reaction of a substituted thiadiazole with *p*-chlorobenzaldehyde in acidic media. The structure of compounds **7** has unambiguously been determined by the use of 1D and 2D NMR data. In the ^{1}H NMR spectrum we observe the peak of the N=CH proton at 10.0–10.6 ppm, which possesses also a stong correlation with the corresponding carbon at 159–152 ppm, as was recorded on the HMQC spectrum. These chemical shifts should correspond to the above-mentioned open chain structure.

Concerning the ^{1}H NMR spectra of the *p*-fluoro substituted compounds **6e** and **7c**, it should be noted that the second-order pattern representative of a *p*-substituted phenyl ring is further complicated due to the presence of the couplings with ^{19}F. A tentative measure of coupling constants gave $J_{H2-F} = 5$ Hz and $J_{H3-F} = 9$ Hz.

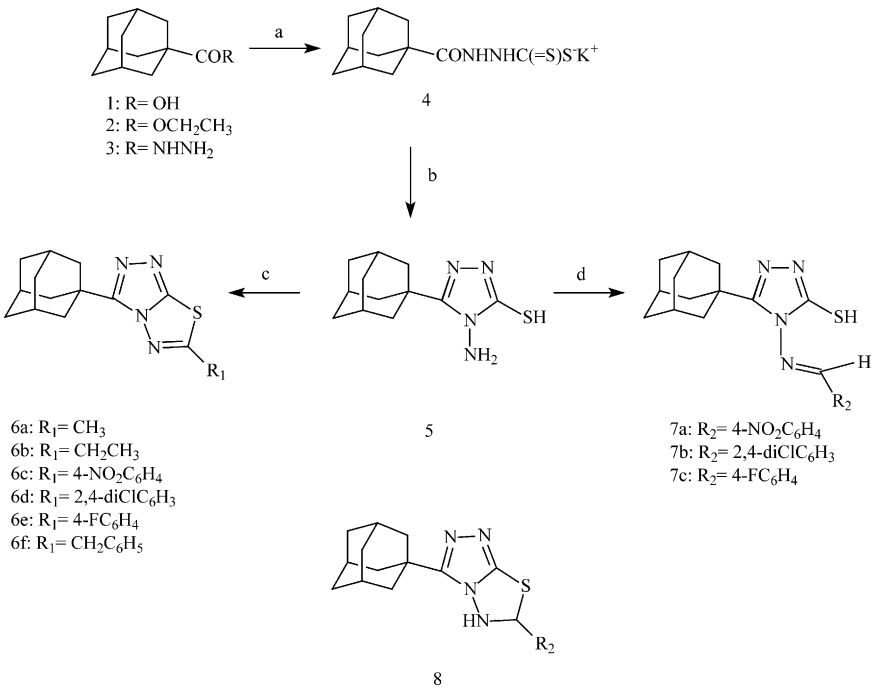
3. Experimental

All reagents used were purchased from Aldrich Chemical Company. Melting points were taken in glass capillary tubes on a Büchi 530 apparatus and are uncorrected. Silica gel TLC was performed on 60_{F-254} precoated sheets and column chromatography was carried out on silica gel (60–120 mesh). IR spectra were recorded on a Perkin–Elmer 833 spectrophotometer. All proton NMR spectra were recorded on a Brucker Avance 400 (400 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard, in chloroform ($CDCl_3$) and chemical shifts were reported as δ (ppm) values. The elemental analyses (C, H, N) of all compounds were performed by the Service Central de Microanalyses (CNRS, Vernaison, France) and are within the range of experimental error ($\pm 0.4\%$ of the calculated values).

*3.1. 6-Methyl-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole (**6a**)*

Acetyl chloride (0.14 ml, 2 mmol) was added to a solution of 5-adamantyl-4-amino-3-mercapto-1,2,4-triazole (**5**, 0.55 g, 2 mmol) [9] in phosphorus oxychloride (10 ml) and the mixture was refluxed for 5 h. The excess of phosphorus oxychloride was then removed under reduced pressure, ice water was added to the residue and the precipitate was filtered, washed with a 20% $NaHCO_3$ solution and water, to give a white solid (530 mg, 88%). M.p.: 216–218 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.75–1.85 (m, 6H, adamantane H), 2.05–2.22 (m, 9H, adamantane H), 2.52 (s, 3H, CH_3). *Anal.* Calc. for $C_{14}H_{18}N_4S$: C, 61.29; H, 6.67; N, 20.42. Found: C, 61.25; H, 6.76; N, 20.36%.

The following compounds were prepared by an analogous procedure.



Scheme 1. (a) CS_2, EtOH, KOH, reflux; (b) NH_2NH_2 95%, reflux; (c) $POCl_3$, R_1COCl, reflux; (d) R_2CHO, EtOH, 70 °C.

3.2. 6-Ethyl-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole (**6b**)

Yield: 78%. M.p.: 211–214 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.4 (t, 3H, $J = 7.4$ Hz, CH_2CH_3), 1.75–1.82 (m, 6H, adamantane H), 2.1–2.25 (m, 9H, adamantane H), 3.0 (q, 2H, $J = 7.5$ Hz, CH_2CH_3). *Anal.* Calc. for $C_{15}H_{20}N_4S$: C, 62.48; H, 6.99; N, 19.43. Found: C, 62.21; H, 6.88; N, 19.20%.

3.3. 6-(4-Nitrophenyl)-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazole (**6c**)

Yield: 84%. M.p.: 280–281 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.8–1.92 (m, 6H, adamantane H), 2.1–2.3 (m, 9H, adamantane H), 8.1 (d, 2H, $J = 9$ Hz, Ar–H), 8.45 (d, 2H, $J = 9$ Hz, Ar–H). *Anal.* Calc. for $C_{19}H_{19}N_5O_2S$: C, 59.83; H, 5.02; N, 18.36. Found: C, 59.71; H, 5.23; N, 18.12%.

3.4. 6-(2,4-Dichorophenyl)-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazole (**6d**)

Yield: 75%. M.p.: 254–256 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.77–1.92 (m, 6H, adamantane H), 2.05–2.25 (m, 9H, adamantane H), 7.50 (dd, 1H, $J =$ 8.5 Hz, 2.1 Hz, 5-ArH), 7.65 (d, 1H, $J = 2.1$ Hz, 3-ArH), 7.94 (d, 1H, $J = 8.5$ Hz, 6-ArH). *Anal.* Calc. for $C_{19}H_{18}Cl_2N_4S$: C, 56.24; H, 4.47; N, 13.81. Found: C, 56.49; H, 4.43; N, 13.68%.

3.5. 6-(4-Fluorophenyl)-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazole (**6e**)

Yield: 72%. M.p.: 224–226 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.75–1.9 (m, 6H, adamantane H), 2.05–2.3 (m, 9H, adamantane H), 7.15 ('deceptively' simple triplet, 2H, $J = 9$ Hz, 3,5-ArH), 7.95 ('deceptively' simple quartet, 2H, $J = 9$ Hz, 5 Hz, 2,6-ArH). *Anal.* Calc. for $C_{19}H_{19}FN_4S$: C, 64.38; H, 5.40; N, 15.81. Found: C, 64.18; H, 5.27; N, 15.72%.

3.6. 6-Benzyl-3-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazolo[3,4-b] [1,3,4]thiadiazole (**6f**)

Yield: 69%. M.p.: 186–187 °C (ethanol). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.75–1.90 (m, 6H, adamantane H), 2.1–2.3 (m, 9H, adamantane H), 4.3 (s, 2H, CH_2Ar), 7.25–7.45 (m, 5H, ArH). *Anal.* Calc. for $C_{19}H_{22}N_4S$: C, 67.43; H, 6.55; N, 16.56. Found: C, 67.21; H, 6.30; N, 16.64%.

3.7. 3-Mercapto-4-(4-nitrobenzylidene)-5-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazole (**7a**)

4-Nitrobenzaldehyde (75 mg, 0.5 mmol) was added to a solution of 5-adamantyl-4-amino-3-mercapto-1,2,4-triazole (**5**, 160 mg, 0.45 mmol) in ethanol (6 ml). The pH was then adjusted to pH 5–6 with diluted HCl and the mixture was heated at 70 °C for 12 h, allowed to stand overnight and the precipitate was filtered, washed with a 5% $NaHCO_3$ solution and water and air-dried. The crude product was then purified by column chromatography on silica gel (1.5 × 20 cm) using a mixture of dichloromethane/methanol (95/5) as mobile phase, to yield pure **7a** as a light yellow crystalline product (130 mg, 53%). M.p.: 271–273 °C (ethyl acetate/hexane). IR (Nujol, υ cm^{-1}): 3080 (NH/SH), 1608 (C=N). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.6–1.75 (m, 6H, adamantane H), 2.05–2.22 (m, 9H, adamantane H), 8.05 (d, 2H, $J = 9.2$ Hz, Ar–H), 8.35 (d, 2H, $J = 9.2$ Hz, Ar–H), 10.50 (s, 1H, N=CH), 10.75 (br. s. 1H, D_2O exchangeable, SH). *Anal.* Calc. for $C_{19}H_{20}N_5O_2S$: C, 59.68; H, 5.27; N, 18.31. Found: C, 59.81; H, 5.33; N, 18.60%.

The following compounds were prepared by an analogous procedure

3.8. 4-(2,4-Dichlorobenzylidene)-3-mercapto-5-(tricyclo[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazole (**7b**)

Yield: 48%. M.p.: 239–241 °C (ethyl acetate/hexane). IR (Nujol, υ cm^{-1}): 3087 (NH/SH), 1610 (C=N). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.6–1.9 (m, 6H, adamantane H), 2–2.2 (m, 9H, adamantane H), 7.37 (dd, 1H, $J = 8.8$ Hz, 2.2 Hz, 5-ArH), 7.49 (d, 1H, $J = 2.2$ Hz, 3-ArH), 8.07 (d, 1H, $J = 8.8$ Hz, 6-ArH), 10.6 (s, 1H, N=CH), 11.15 (br. s. 1H, D_2O exchangeable, SH). *Anal.* Calc. for $C_{19}H_{20}Cl_2N_4S$: C, 56.02; H, 4.95; N, 13.75. Found: C, 55.83; H, 4.66; N, 13.51%.

Table 1
Anti-HIV-1 and HIV-2 activity and cytotoxic properties in MT-4 cells

Compound	EC_{50} [a] (μg/ml)		CC_{50} [b] (μg/ml)
	IIIB	ROD	
6a	>62.1	>62.1	62.1 ± 8.0
6b	>11.2	>11.2	11.2 ± 0.9
6c	>24.0	>24.0	24.0 ± 14.2
6d	>125	>125	>125
6e	>78.0	>78.0	78.0 ± 5.7
6f	>19.1	>19.1	19.1 ± 4.8
7a	>56.5	>56.5	56.5 ± 8.9
7b	>11.7	>11.7	11.7 ± 0.3
7c	>10.9	>10.9	10.9 ± 0.9
Zidovudine	0.0009 ± 0.0003	0.0006 ± 0.0004	18.2 ± 12.3

[a] 50% effective concentration, or concentration required to inhibit the cytopathic effect of HIV in MT-4 cells by 50%.

[b] 50% cytotoxic concentration, or concentration required to reduce the viability of MT-4 cells by 50%.

3.9. 4-(4-Fluorobenzylidene)-3-mercapto-5-(tricyclo-[3,3,1,1^{3,7}]decan-1-yl)-1,2,4-triazole (**7c**)

Yield: 45%. M.p.: 248–250 °C (ethyl acetate/hexane). IR (Nujol, v cm^{-1}): 3092 (NH/SH), 1606 (C=N). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.6–1.85 (m, 6H, adamantane H), 2.05–2.25 (m, 9H, adamantane H), 7.20 ('deceptively' simple triplet, 2H, $J = 8.8$ Hz, 3,5-ArH), 7.88 ('deceptively' simple quartet, 2H, $J = 8.8$ Hz, 5.5 Hz, 2,6-ArH), 10.0 (s, 1H, N=CH), 11.7 (br. s. 1H, D_2O exchangeable, SH). *Anal.* Calc. for $C_{19}H_{21}FN_4S$: C, 64.02; H, 5.94; N, 15.72. Found: C, 64.11; H, 6.17; N, 15.59%.

4. Results and discussion

Compounds were tested for antiviral activity and cytotoxicity in various viral test systems, according to previously published procedures [11,12]. The results of in vitro screening are summarized in Tables 1–4. All compounds were inactive against the replication of HIV-1(III_B) and HIV-2(ROD) at subtoxic concentrations in acutely infected MT-4 cells whereas most of the compounds were cytotoxic for the host cells (Table 1). However, none of the compounds inhibited vesicular stomatitis virus, Coxsackie virus, respiratory syncytial virus, parainfluenza-3 virus, reovirus, Sindbis virus,

Table 2
Cytotoxicity and antiviral activity in HeLa cell cultures

Compound	MCC [a] (μg/ml)	MIC [b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
6a	>400	>400	>400	>400
6b	>400	>400	240	>400
6c	=80	>16	>16	>16
6d	>400	>400	>400	>400
6e	>400	>400	>400	>400
6f	>400	>400	>400	>400
7a	≥80	>16	>16	>16
7b	80	>16	>16	>16
7c	80	>16	>16	>16
BVDU	>400	>400	>400	>400
(S)-DHPA	>400	48	>400	>400
Ribavirin	>400	9.6	48	3.2

[a] Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.
[b] Minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathicity by 50%.

Table 3
Cytotoxicity and antiviral activity in Vero cell cultures

Compound	MCC [a] (μg/ml)	MIC [b] (μg/ml)				
		Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
6a	≥400	>80	>80	>80	>80	>80
6b	≥400	>80	>80	>80	>80	>80
6c	80	>16	>16	>16	>16	>16
6d	≥400	>80	>80	>80	>80	>80
6e	≥400	>80	>80	>80	>80	>80
6f	>400	>400	>400	>400	>400	>400
7a	≥16	>16	>16	>3.2	>3.2	>3.2
7b	≥16	>16	>16	>16	>16	>16
7c	≥16	>16	>16	>3.2	>3.2	>3.2
BVDU	>400	>400	>400	>400	>400	>400
(S)-DHPA	>400	240	240	>400	>400	>400
Ribavirin	>400	48	80	240	240	48

[a] Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.
[b] Minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathicity by 50%.

Table 4
Cytotoxicity and antiviral activity of in E_6SM cell cultures

Compound	MCC [a] (μg/ml)	MIC [b] (μg/ml)					
		HSV-1 (KOS)	HSV-2 (G)	Vaccinia virus	Vesicular stomatitis virus	HSV-1 TK$^-$KOS ACV^r	HSV-1 TK$^-$VMW 1837
6a	400	>80	>80	>80	>80	>80	>80
6b	≥80	>80	>80	>80	>80	>80	>80
6c	80	>16	>16	>16	>16	>16	>16
6d	>400	>400	>400	>400	>400	>400	>400
6e	80	>16	>16	>16	>16	>16	>16
6f	400	>80	>80	>80	>80	>80	>80
7a	80	>16	>16	>16	>16	>16	>16
7b	80	>16	>16	>16	>16	>16	>16
7c	80	>16	>16	>16	>16	>16	>16
BVDU	400	0.0768	80	0.64	>80	>80	16
Ribavirin	>400	400	240	48	48	240	240
ACG	400	0.384	0.384	>80	>80	>80	1.92
DHPG	100	0.096	0.096	>100	>100	>100	0.096

[a] Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.
[b] Minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathicity by 50%.

Punta Toro virus, herpes simplex virus type 1 and 2, and vaccinia virus-induced cytopathicity at subtoxic concentrations in HeLa, Vero or E_6SM cell culture (Tables 2–4).

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